

Urchin Gonad Response to Kelp Forest Restoration on the Palos Verdes Peninsula, California

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Abstract.—Along the Palos Verdes Peninsula in southern California, high densities of *Strongylocentrotus purpuratus* (purple sea urchin) have consumed almost all macroalgae on large expanses (61 ha) of rocky reef habitat, creating “urchin barrens.” *Mesocentrotus franciscanus* (red sea urchin) harvesting comprises an important fishery in the region, as their gonads are sold as a high-value sushi product called “uni.” However, with a lack of macroalgal food resources, urchins in barrens are smaller and exist in a starved state, meaning little, if any, gonad product is available to the fishery. To restore local kelp forests and increase gonad biomass available to the *M. franciscanus* fishery, beginning in October 2013, *S. purpuratus* were culled in barrens to a target density of 2 per m² across 5.2 ha of rocky reef on the Palos Verdes Peninsula. *Mesocentrotus franciscanus* were collected from urchin barren, restoration, and kelp reference sites from April to November 2014 to compare differences in gonad production among the three site types. Culling *S. purpuratus* resulted in the recovery of normal seasonal *M. franciscanus* gonad production throughout the 8-month study. *Mesocentrotus franciscanus* gonad weights at a given test diameter length in restoration sites were equivalent to, and sometimes exceeded, the gonad production of those from the kelp reference sites. The urchin test length distributions of collected *M. franciscanus* were consistently smaller at urchin barren sites than at kelp reference sites, while those in restoration sites generally fell in between.

Giant kelp (*Macrocystis pyrifera*) forests are among the most productive and diverse ecosystems in the world (Dayton 1985; Graham 2004). Kelps are autogenic engineers, providing physical structure for a high diversity of flora and fauna. In addition, they are a food source for a wide variety of taxa, contributing to the food web through direct grazing and as dissolved organic materials (Graham 2004; Duarte et al. 2022). Yet, the combined effects of overfishing, pollution, and increasing frequency of warm water events have led to the destructive grazing of kelp by sea urchins and the formation of “urchin barrens” that can last decades (Steneck et al. 2002; Cavanaugh et al. 2019; Rogers-Bennett and Catton 2019; Kawamata and Taino 2021) (Fig. 1). These barren reefs are largely devoid of macroalgae and covered instead by high proportions of bare rock and encrusting coralline algae

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Fig. 1. (A) High densities of *S. purpuratus* in an urchin barren state, (B) kelp forest state in southern California, (C) partially dissected *Mesocentrotus franciscanus* from a kelp forest site, and (D) extracted *M. franciscanus* urchin gonad (i.e., uni).

(Dayton 1985; Steneck et al. 2002; Cavanaugh et al. 2011; Rogers-Bennett and Catton 2019; Gizzi et al. 2021; Williams et al. 2021).

Feedback mechanisms on temperate rocky reefs increase the resilience of both healthy kelp forest and urchin barren states (Levin and Lubchenco 2008; Baskett and Salomon 2010; Filbee-Dexter and Scheibling 2014). The kelp forest state is maintained by positive feedback mechanisms that prevent high densities of urchins from forming and destructively grazing (Baskett and Salomon 2010; Filbee-Dexter and Scheibling 2014). In the presence of abundant predators, urchins exhibit cryptic behavior and lower densities are maintained (Nichols et al. 2015). However, overfishing of urchin predators can reduce top-down control of urchin abundance, leading to a phase shift where sea urchins have a greater impact on the ecosystem dynamics (Jackson et al. 2001; Steneck et al. 2002; Filbee-Dexter and Scheibling 2014; Melis et al. 2019). When urchin densities subsequently increase above a critical threshold, the transition from a kelp forest to an urchin barren occurs rapidly, resulting in destructive grazing and prevention of substratum growth (Baskett and Salomon 2010; Filbee-Dexter and Scheibling 2014; Karatayev and Baskett 2020; Kawamata and Taino 2021). Evidence from various studies show a phase shift threshold from an algal dominated system to barren formation occurs when urchin biomass exceeds 700 g/m^2 , though exact numbers are highly dependent on region specific dynamics (Ling et al. 2015). An urchin barren state is then maintained by positive feedback mechanisms that promote sea urchin recruitment, settlement, and overgrazing, all of which inhibit kelp settlement (Filbee-Dexter and Scheibling 2014; Sangil and Hernández 2022).

Destructive sea urchin grazing is the leading cause of kelp deforestation in the world (Steneck et al. 2002) and has multi-trophic level impacts on species' use of kelp forest habitat resources (Graham 2004; Rogers-Bennett and Catton 2019). Urchin barrens occur globally in most regions where kelp forests exist (Steneck et al. 2002; Gagnon et al. 2004; Ling et al. 2015). In southern California, urchin barrens have been present on the Palos Verdes Peninsula since the 1950s (North 1963; Foster and Schiel 2010). Surveys conducted in the late 1960s had described a near total absence of adult giant kelp on the Palos Verdes Peninsula (Wilson et al. 1977; Foster and Schiel 2010) likely due to the combined influence of increased coastal development, sedimentation, urban runoff, pollution, and direct kelp removal from storms (North 1963; Dayton 1985; Steneck et al. 2002; Ford and Meux 2010; Foster and Schiel 2010). Following this large-scale kelp reduction, a transition to an urchin dominated system further prevented kelp recruitment (North 1963; Foster and Schiel 2010; Filbee-Dexter and Scheibling 2014). As of 2012, there were 61 ha of rocky reef persisting in an urchin barren state on the Palos Verdes Peninsula (Claisse et al. 2013).

Kelp forest loss and the resulting urchin barrens have systemic ecological implications, as well as significant economic impacts on sea urchin fisheries (Claisse et al. 2013; Rogers-Bennett and Catton 2019). Historically, the *Mesocentrotus franciscanus* (red sea urchin) fishery was consistently one of the largest fisheries in California by annual tonnage harvested (NMFS 2018). *Mesocentrotus franciscanus* are harvested for their gonads, as a high value sushi product called “uni” (Rogers-Bennett 2007; Teck et al. 2018). In urchin barrens, particularly when coexisting with high densities of *S. purpuratus*, *M. franciscanus* gonads are substantially underdeveloped (Harrold and Reed 1985; Kato and Schroeter 1985; Rogers-Bennett et al. 1995; Spindel et al. 2021), resulting in a decreased amount of gonad product available to the fishery (Claisse et al. 2013). For example, in northern California, the *M. franciscanus* fishery remained stable from 2000-2014, but recent extensive losses of kelp, combined with increases in *S. purpuratus* dominated urchin barrens, resulted in urchin gonad biomass declines that eventually led to the collapse of the *M. franciscanus* commercial fishery (Rogers-Bennett and Catton 2019; Angwin et al. 2022).

In an urchin barren state, urchin gonad biomass is reduced due to the lack of macroalgal food available (Bernard 1977; Rogers-Bennett 2007). Other urchin health metrics, including growth rates, test diameter size, density, and biomass, can also be negatively affected by reductions in food resources (Ebert 1967; Claisse et al. 2013; Teck et al. 2017). In addition to using their gonads for reproduction, urchins also use gonads to store nutrients as fats and carbohydrates within the tissue (Doezema and Phillips 1970), having the ability to re-sorb their gut and gonad complex for energy (Giese et al. 1966; Pearse et al. 1970; Kato and Schroeter 1985; Rogers-Bennett et al. 1995). With the almost complete absence of macroalgal food availability in barrens, urchins exist in a starved state (Kato and Schroeter 1985) through metabolic depression, which induces morphological changes (Smith and Garcia 2021; Spindel et al. 2021), permitting the urchins to survive by feeding on plankton and diatoms (Pearse et al. 1970; Kato and Schroeter 1985; Hernández et al. 2011). However, most populations of urchins in barrens have poor health, reduced gonads, and smaller test diameter sizes (Pearse et al. 1970; Ling and Johnson 2009; Claisse et al. 2013; Williams et al. 2021).

In kelp forests, urchin gonad development and spawning follow a seasonal pattern (Ebert et al. 1994; Hernández et al. 2011; Teck et al. 2018), although there is high variation even within the same species over relatively small spatial scales (Kato and Schroeter 1985). In California, increased seasonal gonad production typically occurs in late fall or early winter seasons as a direct result of higher drift kelp availability due to natural kelp forest growth

and kelp recovery following winter and spring storms (Cavanaugh et al. 2011; Teck et al. 2018). Spawning typically occurs directly following a period of peak kelp abundance and gonad production when sea urchins are investing energy into developing gonads for reproduction (Ebert et al. 1994; Teck et al. 2018). While an increase in gonad production is an indicator or cue to spawn, there is evidence that gonad development also occurs independently for energy storage, suggesting there are other seasonal cues at play that induce spawning (Hernández et al. 2011). It is therefore necessary to consider both direct food availability and other seasonal influences on gonad production when considering management efforts to maintain both the commercial urchin fishery and the kelp forest state (Teck et al. 2017; 2018), as well as to inform restoration efforts (Claisse et al. 2013).

A variety of restoration techniques have been implemented to try to restore barrens to kelp forests, and most have involved removing the main driver (i.e., sea urchins) of kelp deforestation (Flukes et al. 2012; Eger et al. 2020; 2022; Layton et al. 2020). On the Palos Verdes Peninsula, kelp forest restoration efforts aimed at reducing barren-forming urchin densities have been ongoing since the early 2000s (Ford and Meux 2010; Williams et al. 2021). In this area, the vast majority of urchins in these barrens are *S. purpuratus* (Claisse et al. 2013). Beginning in 2013, commercial urchin harvesters were employed as part of a large-scale kelp restoration effort along the Palos Verdes Peninsula to reduce *S. purpuratus* density to 2 per m² through urchin culling. The commercially important *M. franciscanus* were not culled in an effort to increase production for the local commercial urchin fishery (Claisse et al. 2013).

The present study examines how *M. franciscanus* gonad biomass production responded to the effects of culling *S. purpuratus* in barrens to restore kelp forests along the Palos Verdes Peninsula in 2014, prior to a natural urchin mass mortality event at the end of that year (Williams et al. 2021). We compare changes in *M. franciscanus* gonad biomass over time collected from three site types: kelp forest reference sites, urchin barren sites, and restoration sites following urchin density reduction from active culling. Sea urchin nutrition and gonad production is positively influenced by increases in kelp availability (Pearse et al. 1970; Claisse et al. 2013; Teck et al. 2018). Further, kelp forests have the ability to rebound rapidly following disturbances (Cavanaugh et al. 2011; Williams et al. 2021), suggesting gonad production should also increase rapidly following restoration activities.

Materials and Methods

All project activities occurred on the Palos Verdes Peninsula, located in Los Angeles County, California (Fig. 2). Habitat areas within sites were initially classified as urchin barrens or kelp forests by a previous study (Claisse et al. 2013). The three site types in this study were designated as urchin barren, kelp reference, and restoration. Urchin barrens were identified as areas of rocky reef almost entirely devoid of macroalgae, characterized by high percent cover of bare substrate, encrusting coralline algae, and high densities of *S. purpuratus* (Claisse et al. 2013; Gizzi et al. 2021). Restoration activities occurred at Honeymoon Cove from 29 October 2013, to 7 April 2014, and at Underwater Arch from 10 September 2013, to 5 August 2014 (Table 1). Divers used rock hammers to reduce *S. purpuratus* densities in barrens to a target density of < 2/m². Divers left *M. franciscanus* in place as they were not overabundant and are economically valuable to the urchin fishery (Claisse et al. 2013). Given the large spatial scale of this restoration effort in the region (22.8 ha total target area), and the nature of the labor force (e.g., three commercial urchin

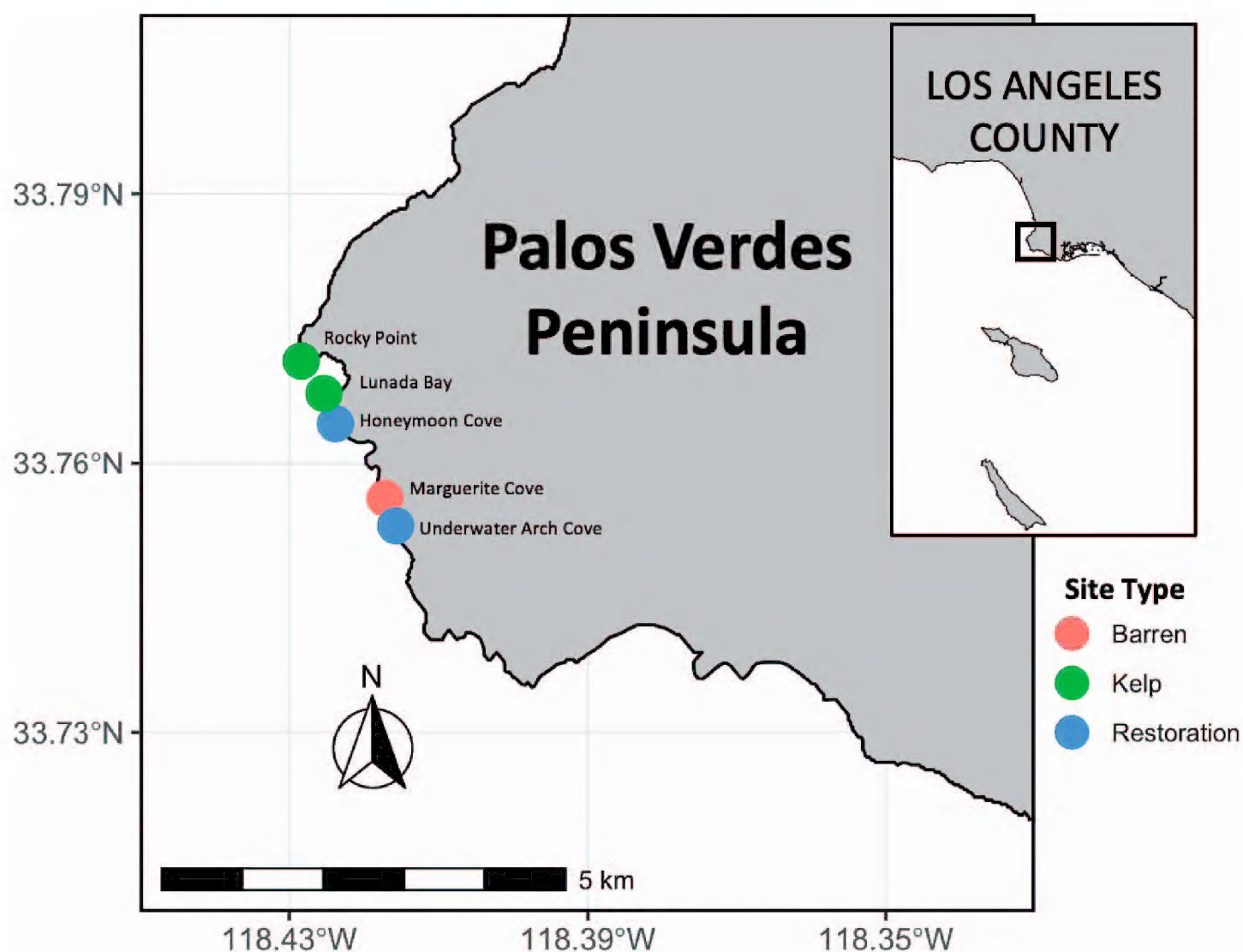


Fig. 2. Urchin survey and collection sites on the Palos Verdes Peninsula, California. Site types are designated by color. Some areas within the restoration sites were used as urchin barren collection sites prior to restoration activities beginning in those areas (Sub-site IDs in Table 1).

diver teams, The Bay Foundation non-profit employees, volunteers), restoration of sites was initially intended to occur sequentially. However, practical considerations resulted in restoration efforts occurring concurrently at some reefs, and divers would often return to sites after a period of weeks to months to monitor and cull additional *S. purpuratus* urchins found in small high-density patches until the entire site was considered ‘restored’ with a target density of $< 2/\text{m}^2$ (restoration end dates listed in Table 1). It is also important to note that *S. purpuratus* densities within most of the restoration areas were typically reduced to the target density months prior to the restoration end date.

In 2013, prior to the start of restoration activities at a given area (Sub-site ID, Table 1), pre-restoration monitoring was conducted at urchin barren sites to be restored per California Department of Fish and Wildlife (CDFW) standards in accordance with the terms of the Scientific Collecting Permit issued to the Bay Foundation (TBF; S-183390001-19133-001). Urchin barren sites were divided into 30 m x 30 m blocks, each comprised of 15 parallel and adjacent (30 m x 2 m) transects. TBF biologists counted *S. purpuratus* along five of the fifteen 30 m x 2 m transects per block to estimate pre-restoration density of *S. purpuratus* of the block (range 57.2 – 21.2/ m^2 ; Table 1).

Post-restoration monitoring was conducted once the team doing the restoration reported to TBF that *S. purpuratus* densities within the block had been reduced to the target density of $< 2/\text{m}^2$. All 15 transects within each 30 m x 30 m block were then surveyed by TBF staff to ensure that no pockets of high-density *S. purpuratus* remained at the site, and if this was

Table 1. *Mesocentrotus franciscanus* collection and restoration activities (i.e., culling *S. purpuratus*). Sub-site ID and associated latitude and longitude give the specific areas for each collection within the overall site location (Site Name): Honeymoon Cove (HMC), Lunada Bay (LB), Underwater Arch Cove (UAC), Marguerite Cove (MC), Rocky Point (RP). Restoration Start is the first day restoration actions began, and Restoration End is the last day restoration actions occurred in a specific Sub-site ID. Pre and Post refers to the *S. purpuratus* density before and after culling in a specific Sub-site ID. n is the number of *M. franciscanus* collected from each location on each collection date.

Collection Date	Site Type	Site Name	Latitude	Longitude	Sub-site ID	Restoration Start	Pre (No./m ²)	Restoration End	Post (No./m ²)	n
29 Apr 2014	Barren	HMC	33.7648	-118.4232	HMC_B1_B	-	-	-	-	47
	Kelp	LB	33.7690	-118.4255	LB_K1_K	-	-	-	-	30
	Rest.	HMC	33.7637	-118.4234	HMC_T1_R	29 Oct 2013	54.4	26 Feb 2014	1.6	49
28 May 2014	Rest.	UAC	33.7521	-118.4157	UAC_J1_R	25 Oct 2013	48.9	11 Apr 2014	2.3	48
	Barren	UAC	33.7539	-118.4156	UAC_B2_B	-	-	-	-	26
	Kelp	LB	33.7691	-118.4246	LB_K2_K	-	-	-	-	37
	Rest.	HMC	33.7637	-118.4234	HMC_T1_R	29 Oct 2013	54.4	26 Feb 2014	1.6	52
	Rest.	HMC	33.7648	-118.4247	HMC_R1_R	01 Nov 2013	42.6	21 Mar 2014	1.6	51
	Rest.	UAC	33.7521	-118.4157	UAC_J1_R	25 Oct 2013	48.9	11 Apr 2014	2.3	50
	Rest.	UAC	33.7541	-118.4163	UAC_3_R	10 Sep 2013	57.2	11 Dec 2013	3.1	62
26 June 2014	Barren	UAC	33.7536	-118.4150	UAC_B3_B	-	-	-	-	36
	Kelp	LB	33.7664	-118.4257	LB_K3_K	-	-	-	-	33
	Rest.	HMC	33.7637	-118.4234	HMC_T1_R	29 Oct 2013	54.4	26 Feb 2014	1.6	38
	Rest.	UAC	33.7521	-118.4157	UAC_J1_R	25 Oct 2013	48.9	11 Apr 2014	2.3	39
22 July 2014	Barren	UAC	33.7525	-118.4148	UAC_B4_B	-	-	-	-	29
	Kelp	LB	33.7680	-118.4256	LB_K4_K	-	-	-	-	50
	Rest.	HMC	33.7650	-118.4250	HMC_R1_R	01 Nov 2013	42.6	21 Mar 2014	1.6	43
	Rest.	UAC	33.7521	-118.4157	UAC_J1_R	25 Oct 2013	48.9	11 Apr 2014	2.3	53
30 Oct 2014	Barren	MC	33.7556	-118.4174	MC_B5_B	-	-	-	-	58
	Kelp	RP	33.7714	-118.4284	RP_K5_K	-	-	-	-	42
	Rest.	HMC	33.7643	-118.4234	HMC_T2_R	11 Mar 2014	21.2	07 Apr 2014	1.9	47
	Rest.	UAC	33.7539	-118.4158	UAC_W1_R	11 Dec 2013	34.6	05 Aug 2014	1.5	60
18 Nov 2014	Barren	MC	33.7560	-118.4166	MC_B6_B	-	-	-	-	55
	Kelp	LB	33.7662	-118.4254	LB_K6_K	-	-	-	-	54
	Rest.	HMC	33.7640	-118.4234	HMC_T1_R	29 Oct 2013	54.4	26 Feb 2014	1.6	55
	Rest.	UAC	33.7522	-118.4154	UAC_J1_R	25 Oct 2013	48.9	11 Apr 2014	2.3	54

the case, then that was recorded as the restoration end date. Post-restoration *S. purpuratus* densities ranged from 3.1 to 1.5/m² across blocks (Table 1).

To examine differences in gonad production between site types over time, TBF staff and volunteers collected a total of 1,198 *M. franciscanus* > 40 mm test diameter from urchin barrens, restoration sites, and kelp reference sites on six sampling dates (29 April, 28 May, 26 June, 22 July, 30 October, and 18 November) throughout 2014. On each collection date, urchins were collected from one urchin barren, one kelp reference, and two restoration sites (Fig. 2, Table 1). Divers attempted to collect at least 30 *M. franciscanus* along a 30 m x 2 m transect using pry bars. They were placed into game bags, brought to the boat, and placed in dry coolers. They were then transported live to Loyola Marymount University’s Seaver Science Center for dissection. Urchin tests were measured to the nearest mm using calipers and urchin weight was measured to the nearest hundredth of a gram. Gonads (Fig. 1) were then removed and weighed to nearest hundredth of gram. All analyses and figures for this study were produced in R (R Core Development Team 2021).

The modeling approach used in this analysis followed Claisse et al. (2013). An allometry model was used to quantify the relationship between mean gonad weight and test diameter length: $G = \alpha(L - 40)^\beta$ (Eq. 1) (Ebert et al. 2011), where G is gonad weight (g), L is test diameter length (mm), 40 mm is the test diameter length at which *M. franciscanus* is able to first produce a gonad and reproduce (Tegner and Dayton 1981; Kato and Schroeter 1985; Tegner 1989), and both α and β are equation constants. Mean gonad weight at test diameter length was fitted to the data using the ‘mle2’ package in R by minimizing the negative log-likelihood and assuming that G follows a lognormal distribution with mean

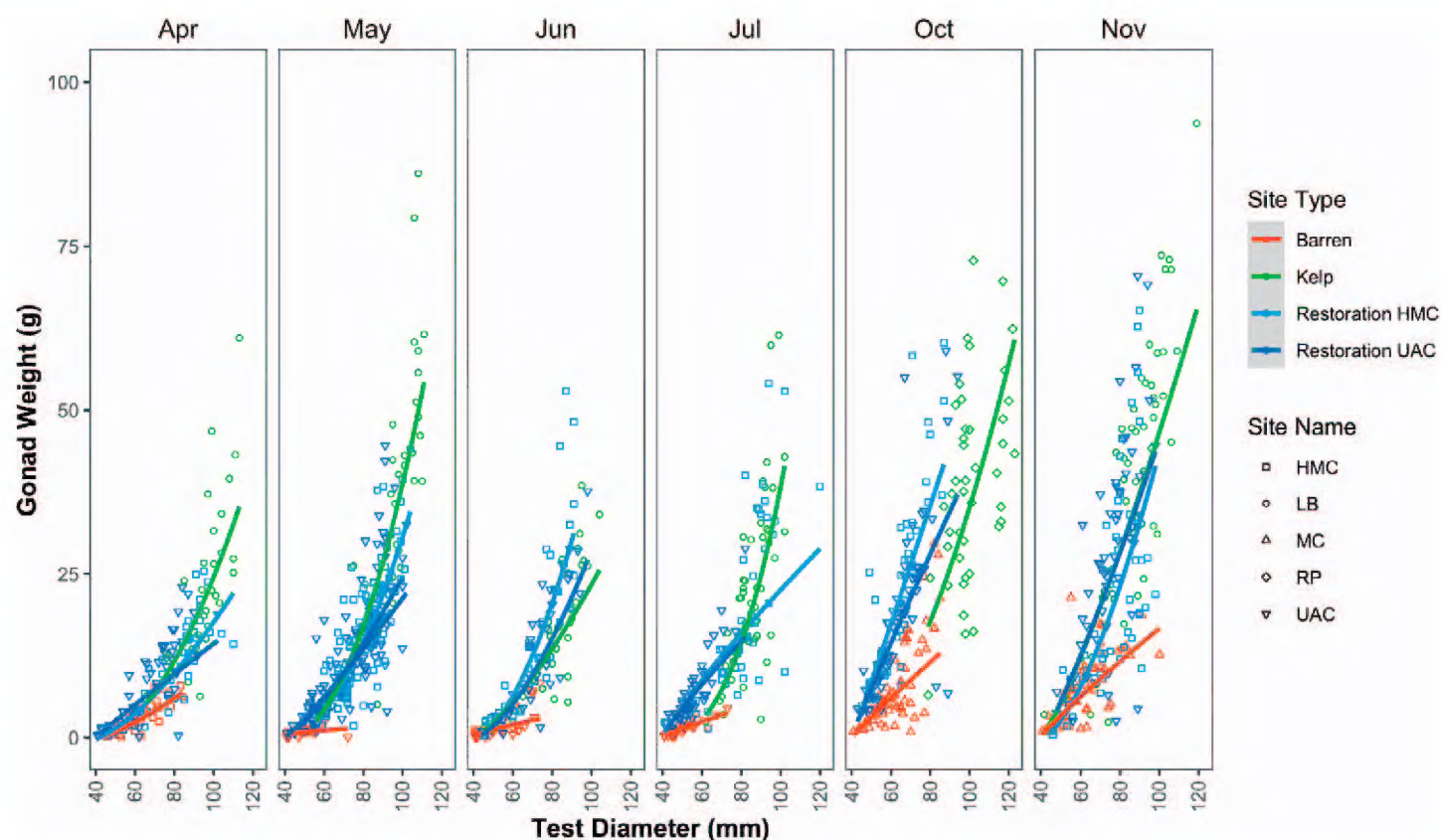


Fig. 3. The relationship between mean gonad weight (g) and test diameter size (mm) of *M. franciscanus* collected from April–November 2014 by the site type (color) and site name (shape): Honeymoon Cove (HMC), Lunada Bay (LB), Underwater Arch Cove (UAC), Marguerite Cove (MC), and Rocky Point (RP). Parameter estimates for each curve are reported in Appendix Table A1 and A2. One point in the November panel from Restoration HMC (gonad weight 121.52 g at 91 mm test diameter) is not shown so to better visualize the y-axis range of most data.

determined by Eq. 1 and the standard deviation of the logarithm (sdlog) (Bolker 2008; Claisse et al. 2013).

In order to account for differences in *M. franciscanus* size structure among urchin barrens, kelp reference, and restoration sites, a bootstrapping approach was used to estimate the 95% confidence intervals to compare differences in mean gonad weight at test diameter length from each site following Haddon (2011) and Claisse et al. (2013). The 95% confidence intervals were then used to assess differences between site types on each collection date, with non-overlapping 95% confidence intervals considered “significant”. In addition to the full model, we also specifically compared 95% CIs for mean gonad weight at test diameter lengths 84 mm and 68 mm. The size of 84 mm was chosen as it is the minimum size limit of *M. franciscanus* in the California urchin fishery, while 68 mm was chosen as being more representative of the size structure within urchin barrens, since most urchins in these sites were < 84 mm.

Results

Mesocentrotus franciscanus urchin gonad weight at a given test diameter in restoration sites was higher than in urchin barrens and similar to kelp reference sites throughout most of the year following the completion of restoration activities (Fig. 3). By May 2014, gonad production in the sampled restoration sites had “recovered,” i.e., mean gonad weight at a given test size was similar to that from kelp sites (Fig. 3), and significantly higher than mean gonad weights at barren sites (Fig. 4, Appendix Table A1, Appendix Fig. A2). A seasonal temporal pattern in this relationship was also present for each site type with gonad weight

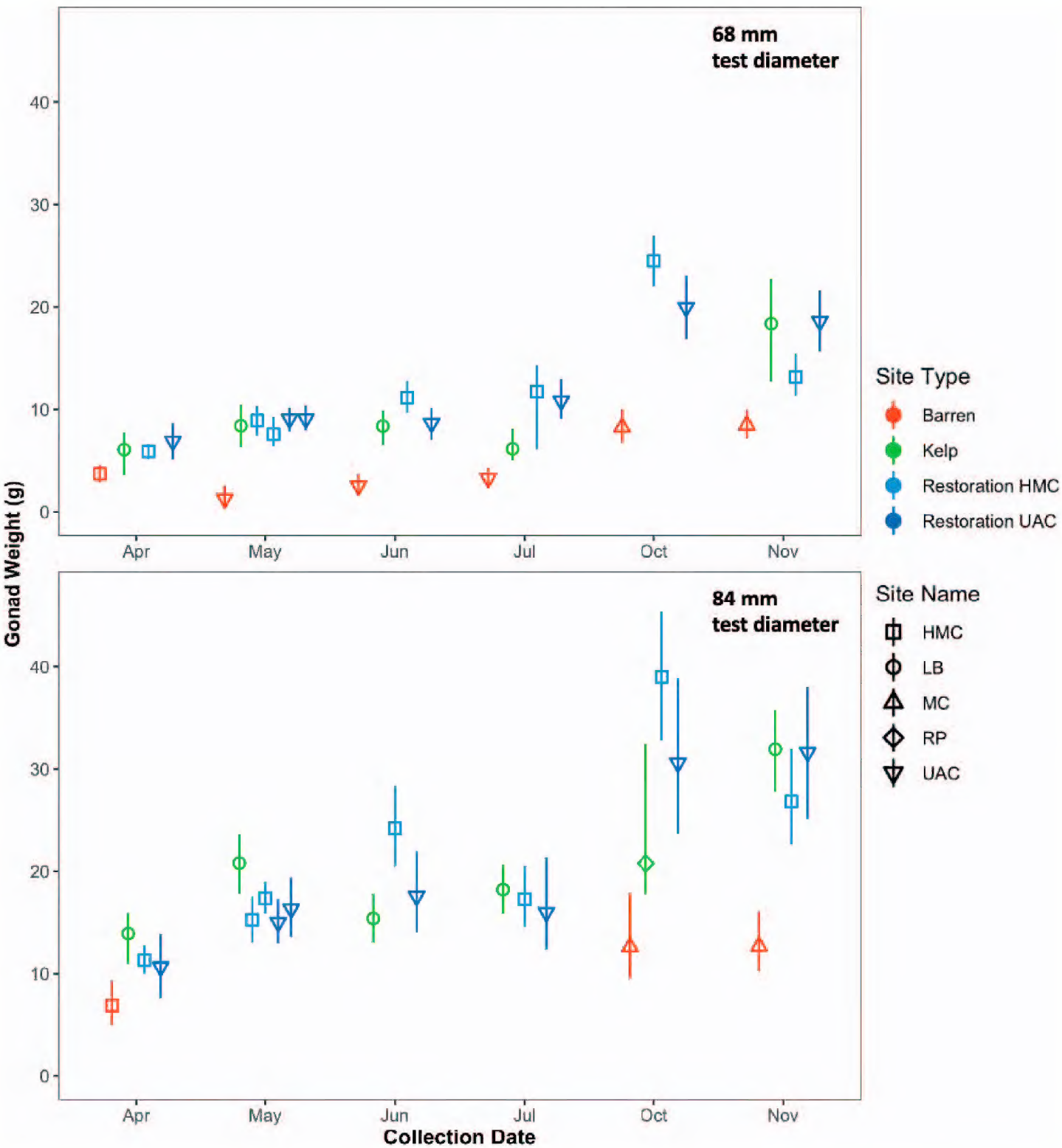


Fig. 4. *Mesocentrotus franciscanus* mean gonad weight (g) with 95% bootstrap confidence interval error bars at 68 mm (top) and 84 mm (bottom) test diameter collected from April–November 2014 by the site type (color) and site name (shape): Honeymoon Cove (HMC), Lunada Bay (LB), Underwater Arch Cove (UAC), Marguerite Cove (MC), and Rocky Point (RP). The size of 84 mm was chosen as it is the minimum size limit of *M. franciscanus* in the California urchin fishery, while 68 mm was chosen as being more representative of the size structure within urchin barrens, since most urchins in these sites were < 84 mm. *Mesocentrotus franciscanus* collected from Rocky Point (kelp reference site) in October 2014 were not included in top panel because all urchin test diameters exceeded 68 mm. *Mesocentrotus franciscanus* collected from Underwater Arch Cove barren sites May–July were not included in the lower panel because all urchin test diameters were less than 84 mm. Mean gonad weights at test diameter length with 95% CIs are reported in Appendix Table A1.

at a given length generally increasing across the April to November sampling period (Fig. 3, Appendix Fig. A1). In April 2014, *M. franciscanus* gonads from restoration sites had an average of 72.5% and 60% larger weight at test diameters 68 mm and 84 mm than those from urchin barrens, respectively. During the peak season (October–November) the average

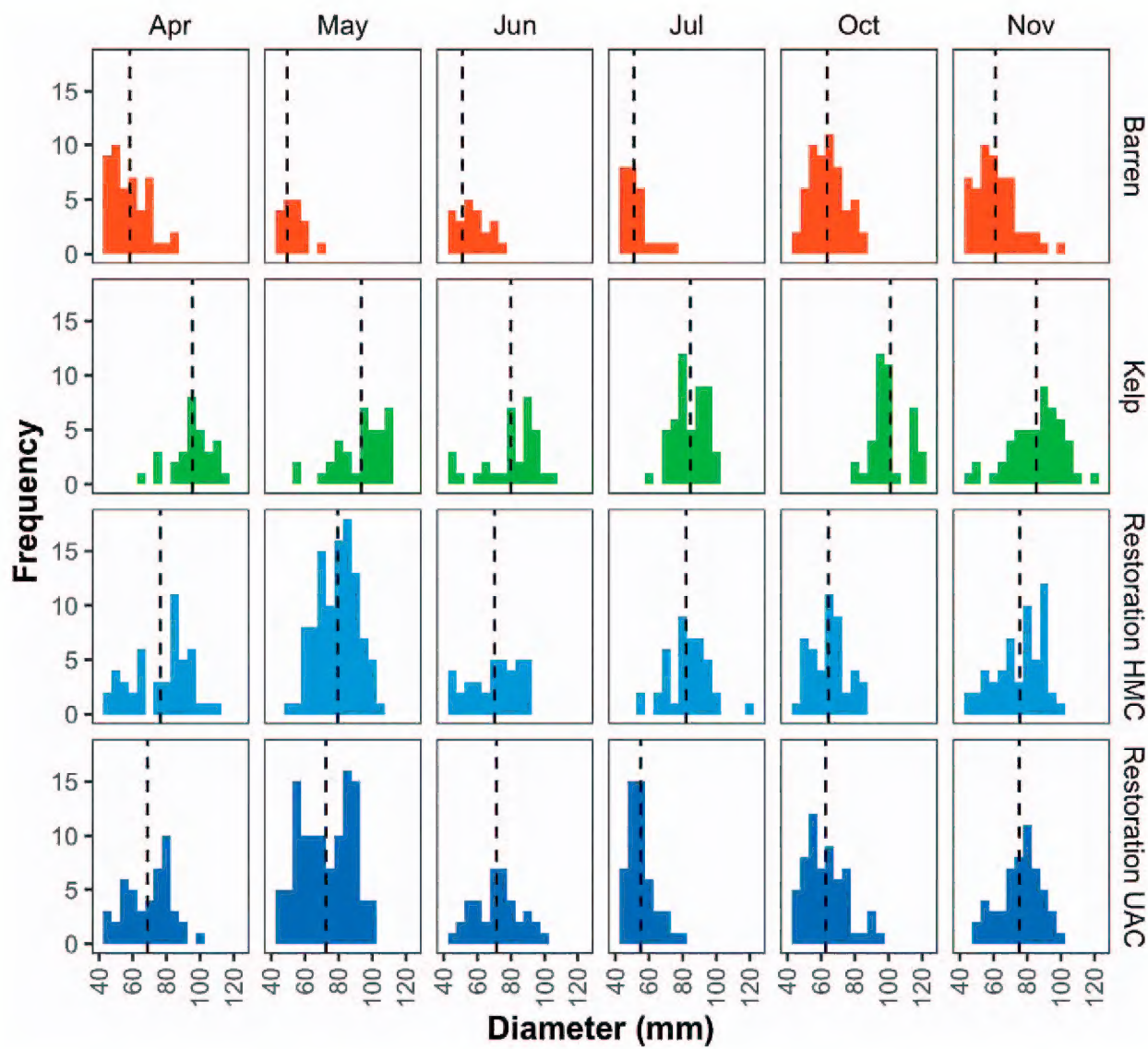


Fig. 5. *Mesocentrotus franciscanus* test diameter (mm) size distribution (5 mm size classes) for urchins collected across April to November 2014 from urchin barrens (red), kelp reference sites (green), and the two restoration sites (blue): Honeymoon Cove (HMC) and Underwater Arch Cove (UAC). Mean lengths are indicated by a vertical dashed line. All *M. franciscanus* less than 40 mm test diameter were removed from analysis, which is the size at which they can first produce a gonad and reproduce (Tegner and Dayton 1981; Kato and Schroeter 1985; Tegner 1989).

gonad weight at 68 mm and 84 mm test diameter at restoration sites increased to 128% and 154% greater than those from urchin barrens, respectively (Fig. 4, Appendix Table A1).

Generally, *M. franciscanus* collected at urchin barren sites had smaller test diameters and those collected at kelp reference sites had larger test diameters, with those from restoration sites falling in between (Fig. 5, Appendix Table A3). In all six data collection months between April–November 2014, *M. franciscanus* collected at kelp reference sites had greater mean test diameter than those collected at urchin barrens and restoration sites. In five of the six data collection months, *M. franciscanus* collected at restoration sites had higher mean test diameters than those collected at urchin barrens.

Discussion and Conclusions

Reducing *S. purpuratus* density in urchin barrens on nearshore rocky reefs along the Palos Verdes Peninsula to the target density of 2/m² through active culling resulted in the recovery of normal seasonal *M. franciscanus* gonad production throughout the 8 mos sampled following completion of restoration activities. Gonad weight at a given length from urchin in restoration sites matched, and sometimes exceeded, the gonad production

of urchin in kelp reference sites. For *M. franciscanus* collected throughout 2014, test diameter, gonad weight relative to test diameter, and gonad weight at a given test diameter in restoration sites were higher than in urchin barrens and similar to those in kelp reference sites. Even though a seasonal effect of increased gonad production was apparent at all site types throughout the year, in restoration sites, *M. franciscanus* gonad weight of legal-size urchins (84 mm test diameter) were 154% higher than in urchin barrens during October and November when the largest gonads weights were observed. Claisse et al. (2013) estimated the potential effects of restoration on gonad biomass by reporting differences between urchin barrens and kelp reference sites indicating restoration could potentially increase gonad biomass available to the commercial urchin fishery. The present study expands on those findings by also assessing urchins in restoration sites relative to urchin barrens and kelp reference sites using the same methods. These results build upon a concurrent study on the Palos Verdes Peninsula, which concluded that declines in urchin density initiated a quick recovery of kelp dominated state in approximately 6 mos (Williams et al. 2021), a timeline consistent with the results of other urchin reduction kelp restoration studies (Sangil and Hernández 2022).

A seasonal pattern was evident in *M. franciscanus* as gonad weight relative to test diameter size increased from the earlier to later months in 2014 across all three site types. This pattern has been observed elsewhere in southern California, as sea urchin spawning normally occurs in the winter and early spring after an annual peak in gonad size is reached in the late fall, although there is some variability among geographic areas (Kato and Schroeter 1985; Ebert et al. 1994; Teck et al. 2018). These seasonal increases in gonad development coincide with natural patterns of kelp growth and recovery following winter and spring storms (Cavanaugh et al. 2011; Teck et al. 2018). Spawning then occurs right after peak gonad production when kelp becomes less abundant (Teck et al. 2018). Therefore, increases in gonad weight relative to test diameter size at all three site types in our study was likely due to natural growth of kelp in the summer and fall months. In restoration sites, gonad weight relative to test diameter increased in accordance with this seasonal pattern but at an even greater magnitude than kelp reference sites and urchin barren sites, indicative of the additional effect of restoration on gonad production. Interestingly, urchin barrens also exhibited a low level of seasonal increase on gonad production, even though these sites maintained an almost complete lack of macroalgae (Williams et al. 2021). This is expected considering natural increases in macroalgae abundance over this period (Cavanaugh et al. 2011; Teck et al. 2018) would result in increased drift algae availability across all site types, in turn, increasing gonad production even at barren sites (Rogers-Bennett et al. 1995; Britton-Simmons et al. 2012).

Claisse et al. (2013) determined that although *M. franciscanus* densities were far greater in urchin barrens than in kelp forests, the lack of gonad production in urchins from barrens resulted in gonad biomass in kelp reference sites greatly exceeding the overall biomass from urchin barrens. However, collections from their study were made in April-May 2011, prior to the peak season of gonad development (Claisse et al. 2013; Teck et al. 2018). When comparing mean gonad weight at 84 mm test diameter, the minimum size limit for the fishery, between urchin barrens and restored sites, we found the difference increased from 60% greater in April to 154% greater during the peak season (October-November). This suggests that the estimates made by Claisse et al. (2013) that restoration could potentially result in an approximately 900% increase in *M. franciscanus* gonad biomass available to the fishery were likely conservative, and the fishery benefits of restoration may be substantially higher.

The test size distributions of collected *M. franciscanus* were significantly smaller at urchin barren sites than at kelp reference sites, while those in restoration sites generally fell in between [$p < 0.001$; mean test diameter (95% CI); Kelp 90 mm (84 – 96), Barren 56 mm (50 – 62), Restoration HMC 75 mm (69 – 81), Restoration UAC 68 mm (62 – 74); Fig. 5]. These results are consistent with Claisse et al. (2013) who found urchins collected in kelp reference sites had mean test diameters that were approximately 50% larger than those in urchin barrens. Similarly, Williams et al. (2021) found that average test lengths of *S. purpuratus* were also lower urchin barrens but increased following recovery to kelp forest conditions.

Resource management strategies that maintain urchin predator abundance, such as marine protected areas, are beneficial to maintaining stable kelp forests (Kawamata and Taino 2021), however, additional adaptive management actions are likely necessary beyond establishing marine reserves if urchin barrens are extensive (Levin and Lubchenco 2008; Baskett and Salomon 2010; Bonaviri et al. 2011; Claisse et al. 2013; Gizzi et al. 2021; Miller et al. 2022). While marine reserves can increase urchin predator populations such as the California spiny lobster (*Panulirus interruptus*) and California Sheephead (*Bodianus pulcher*) (Teck et al. 2017), this recovery can take more than fifteen years after implementation of protection and fishing ceases (Malakhoff and Miller 2021). Further, Eurich et al. (2014) experimentally demonstrated that California spiny lobster would actively select *S. purpuratus* from kelp forests over those from barrens, likely due to their diminished nutritional capacity with a lack of gonad tissue. Accordingly, the recovery of gonad biomass production observed in our study after culling *S. purpuratus* should accelerate the return of these important trophic pathways, ultimately increasing the resilience of restored kelp forests. Quantifying gonad production in sea urchins is an important measure of ecological function in kelp forest ecosystems and should be used to inform adaptive management of restoration projects.

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Appendix Table A1. Model parameter estimates of *M. franciscanus* mean gonad weight at test diameter 84 mm and 68 mm with 95% bootstrap CIs in parentheses for urchins collected April–November 2014 from urchin barrens, kelp reference, and restoration sites (Honeymoon Cove (HMC), Lunada Bay (LB), Underwater Arch Cove (UAC), Marguerite Cove (MC), Rocky Point (RP)). *M. franciscanus* collected from Underwater Arch Cove barren sites May–July were removed from this analysis because all urchin test diameters were less than 84 mm. *Mesocentrotus franciscanus* collected from the Rocky Point kelp reference site in October were removed from this analysis because all urchin test diameters exceeded 68 mm.

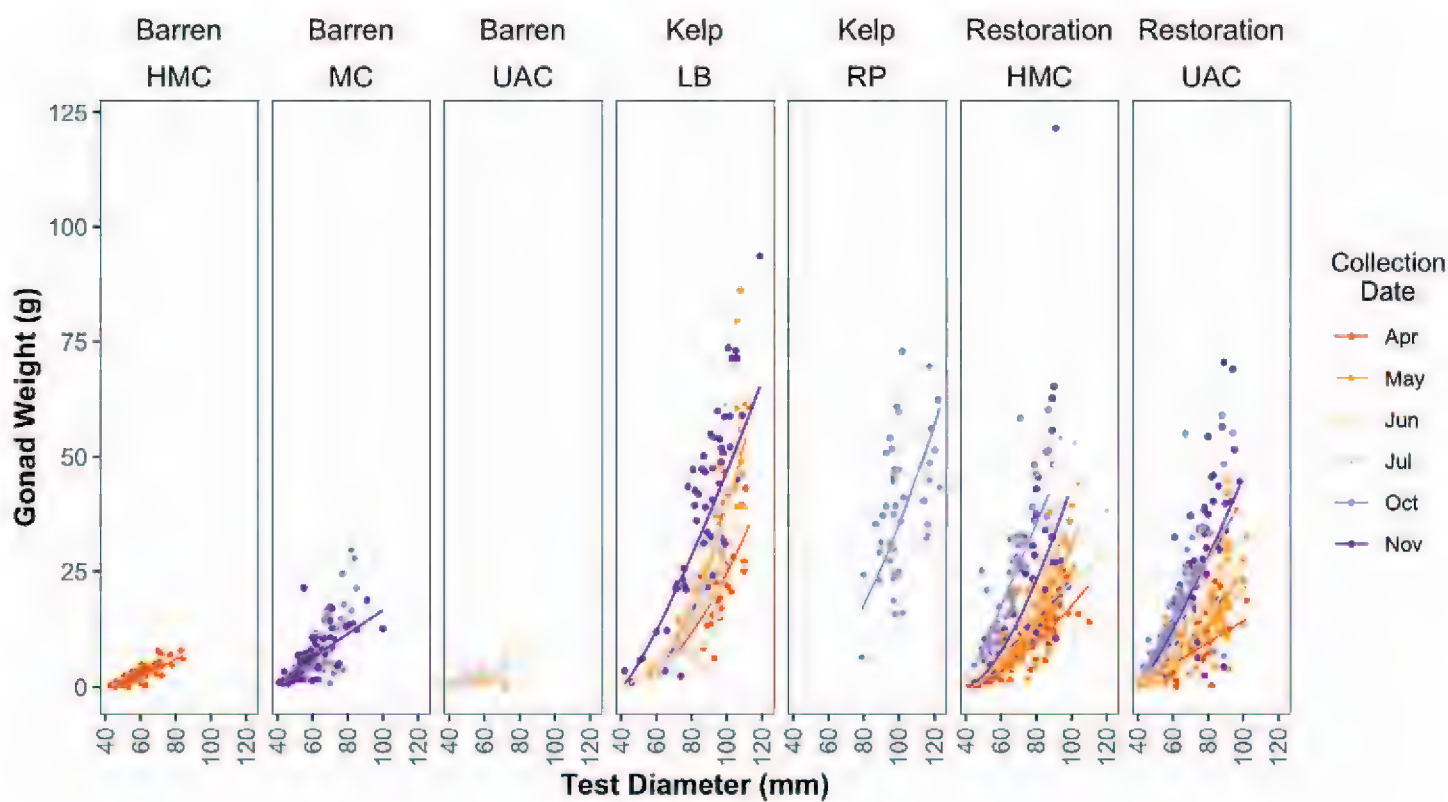
Collection Date	Site Type	Site Name	Site ID	84 mm Test Diameter		68 mm Test Diameter	
				Mean Weight (g)	95% CI	Mean Weight (g)	95% CI
29 Apr 2014	Barren	HMC	HMC_B1_B	6.9	(5–9.3)	3.7	(2.9–4.6)
	Kelp	LB	LB_K1_K	13.9	(11–15.9)	6.1	(3.6–7.8)
	Rest.	HMC	HMC_T1_R	11.3	(10–12.7)	5.9	(5.1–6.6)
	Rest.	UAC	UAC_J1_R	10.7	(7.6–13.9)	6.9	(5.2–8.6)
28 May 2014	Barren	UAC	UAC_B2_B	-	-	1.3	(0.6–2.6)
	Kelp	LB	LB_K2_K	20.8	(17.8–23.6)	8.4	(6.3–10.5)
	Rest.	HMC	HMC_T1_R	15.3	(13.1–17.5)	8.9	(7.5–10.3)
	Rest.	UAC	UAC_J1_R	15.0	(13–17.3)	9.1	(7.9–10.2)
	Rest.	UAC	UAC_2_R	16.3	(13.6–19.4)	9.1	(8–10.4)
	Rest.	HMC	HMC_R1_R	17.4	(15.9–19)	7.6	(6.4–9.2)
26 June 2014	Barren	UAC	UAC_B3_B	-	-	2.5	(1.7–3.7)
	Kelp	LB	LB_K3_K	15.4	(13.1–17.8)	8.4	(6.5–9.9)
	Rest.	HMC	HMC_T1_R	24.2	(20.5–28.3)	11.2	(9.7–12.8)
	Rest.	UAC	UAC_J1_R	17.6	(14.1–21.9)	8.7	(7.1–10.1)
22 July 2014	Barren	UAC	UAC_B4_B	-	-	3.3	(2.6–4.3)
	Kelp	LB	LB_K4_K	18.2	(15.8–20.6)	6.2	(5–8.1)
	Rest.	UAC	UAC_J1_R	16.0	(12.4–21.3)	10.8	(9.1–13)
	Rest.	HMC	HMC_R1_R	17.3	(14.6–20.5)	11.7	(6.1–14.3)
30 Oct 2014	Barren	MC	MC_B5_B	12.6	(9.6–17.8)	8.3	(6.8–10)
	Kelp	RP	RP_K5_K	20.8	(17.8–32.4)	-	-
	Rest.	HMC	HMC_T2_R	39.0	(32.8–45.4)	24.5	(22–26.9)
	Rest.	UAC	UAC_W1_R	30.6	(23.7–38.9)	20.0	(16.9–23)
18 Nov 2014	Barren	MC	MC_B6_B	12.7	(10.2–16.1)	8.5	(7.2–10)
	Kelp	LB	LB_K6_K	31.9	(27.8–35.8)	18.4	(12.7–22.7)
	Rest.	HMC	HMC_T1_R	26.8	(22.6–32)	13.2	(11.3–15.4)
	Rest.	UAC	UAC_J1_R	31.6	(25.1–38)	18.6	(15.6–21.6)

Appendix Table A2. Model parameter estimates of *M. franciscanus* mean gonad weight at test diameter for urchins collected April-November 2014 from urchin barrens, kelp reference, and restoration sites (Hon-eymoon Cove (HMC), Lunada Bay (LB), Underwater Arch Cove (UAC), Marguerite Cove (MC), Rocky Point (RP)).

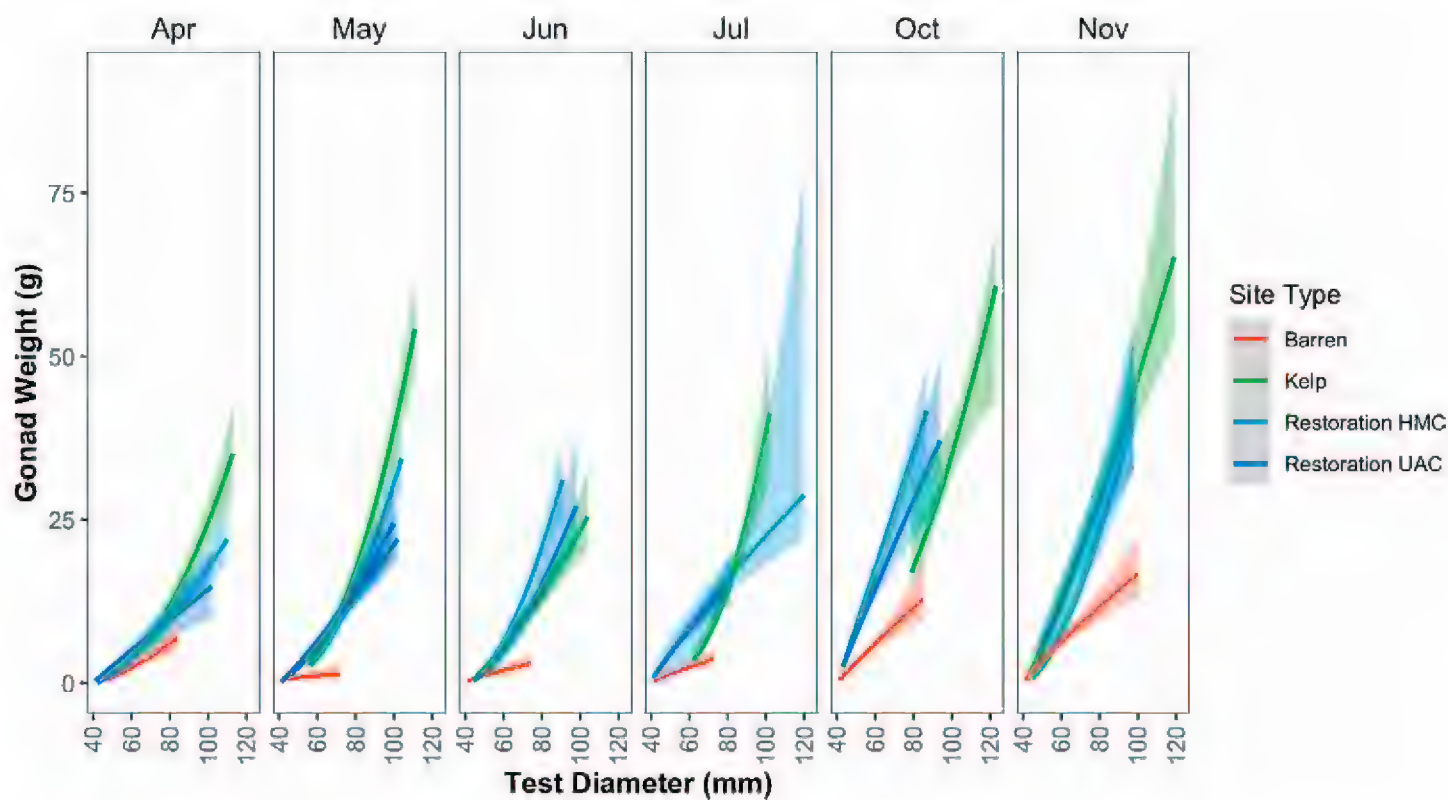
Collection Date	Site Type	Site Name	Site ID	α	β	sdlog
29 Apr 2014	Barren	HMC	HMC_B1_B	0.0400	1.36	0.67
	Kelp	LB	LB_K1_K	0.0136	1.83	0.37
	Rest.	HMC	HMC_T1_R	0.0495	1.44	0.41
	Rest.	UAC	UAC_J1_R	0.2823	0.96	0.86
28 May 2014	Barren	UAC	UAC_B2_B	0.3416	0.40	0.89
	Kelp	LB	LB_K2_K	0.0106	2.00	0.36
	Rest.	HMC	HMC_T1_R	0.1722	1.19	0.47
	Rest.	UAC	UAC_J1_R	0.2211	1.11	0.48
	Rest.	UAC	UAC_2_R	0.1202	1.30	0.53
	Rest.	HMC	HMC_R1_R	0.0170	1.83	0.34
26 June 2014	Barren	UAC	UAC_B3_B	0.2110	0.75	0.84
	Kelp	LB	LB_K3_K	0.0940	1.35	0.45
	Rest.	HMC	HMC_T1_R	0.0369	1.71	0.48
	Rest.	UAC	UAC_J1_R	0.0467	1.57	0.55
22 July 2014	Barren	UAC	UAC_B4_B	0.1876	0.86	0.60
	Kelp	LB	LB_K4_K	0.0021	2.40	0.47
	Rest.	UAC	UAC_J1_R	0.6185	0.86	0.36
	Rest.	HMC	HMC_R1_R	0.6817	0.85	0.60
30 Oct 2014	Barren	MC	MC_B5_B	0.3675	0.93	0.61
	Kelp	RP	RP_K5_K	0.0344	1.69	0.41
	Rest.	HMC	HMC_T2_R	0.7961	1.03	0.36
	Rest.	UAC	UAC_W1_R	0.8570	0.94	0.42
18 Nov 2014	Barren	MC	MC_B6_B	0.4363	0.89	0.56
	Kelp	LB	LB_K6_K	0.3130	1.22	0.53
	Rest.	HMC	HMC_T1_R	0.0692	1.58	0.54
	Rest.	UAC	UAC_J1_R	0.3722	1.17	0.60

Appendix Table A3. *Mesocentrotus franciscanus* collection metadata and descriptive summary statistics for each urchin collections April-November 2014 (Honeymoon Cove (HMC), Lunada Bay (LB), Underwater Arch Cove (UAC), Marguerite Cove (MC), Rocky Point (RP)).

Collection Date	Site Type	Site Name	Sub-site ID	n	Test Diameter (mm)					Gonad Weight (g)				
					Min	Med	Mean	Max	SD	Min	Med	Mean	Max	SD
29 Apr 2014	Barren	HMC	HMC_B1_B	47	43	56	58	84	11	0.00	1.84	2.58	7.92	2.23
	Kelp	LB	LB_K1_K	30	66	97	95	113	11	6.30	22.03	23.53	61.01	12.76
	Rest.	HMC	HMC_T1_R	49	42	83	76	110	17	0.41	10.48	10.20	25.36	7.27
	Rest.	UAC	UAC_J1_R	48	41	72	69	102	15	0.15	9.24	8.90	22.98	6.31
28 May 2014	Barren	UAC	UAC_B2_B	26	41	49	49	72	8	0.00	0.88	1.01	4.15	0.89
	Kelp	LB	LB_K2_K	37	56	96	93	111	15	2.29	35.70	34.78	86.16	19.94
	Rest.	HMC	HMC_T1_R	52	52	75	75	94	9	1.79	12.60	12.98	25.97	6.39
	Rest.	HMC	HMC_R1_R	51	57	87	84	104	12	1.88	17.72	18.82	44.02	10.19
	Rest.	UAC	UAC_J1_R	50	41	75	73	102	17	0.71	11.11	12.88	38.12	10.03
26 June 2014	Rest.	UAC	UAC_2_R	62	45	71	72	100	15	0.67	9.99	12.48	44.64	9.73
	Barren	UAC	UAC_B3_B	36	41	47	51	74	11	0.00	1.00	1.60	8.36	2.06
	Kelp	LB	LB_K3_K	33	45	83	80	104	16	0.71	15.48	15.65	38.47	10.42
	Rest.	HMC	HMC_T1_R	38	45	72	70	91	15	0.19	12.19	15.85	52.91	13.66
	Rest.	UAC	UAC_J1_R	39	44	72	71	98	13	0.64	9.61	12.71	37.60	9.55
22 July 2014	Barren	UAC	UAC_B4_B	29	41	50	51	73	8	0.13	1.54	1.61	4.47	1.15
	Kelp	LB	LB_K4_K	50	62	85	85	102	9	2.45	20.20	21.86	61.44	13.24
	Rest.	HMC	HMC_R1_R	43	41	83	82	120	14	1.36	15.37	19.91	54.10	13.59
	Rest.	UAC	UAC_J1_R	53	42	54	55	82	9	0.95	5.26	6.81	28.85	5.18
30 Oct 2014	Barren	MC	MC_B5_B	58	41	64	63	85	11	0.86	5.68	8.51	29.62	6.87
	Kelp	RP	RP_K5_K	42	79	98	101	123	11	6.48	38.34	39.14	72.82	14.88
	Rest.	HMC	HMC_T2_R	47	45	65	64	87	11	4.83	20.03	22.87	60.27	14.11
	Rest.	UAC	UAC_W1_R	60	43	61	63	94	12	3.48	12.96	17.85	59.04	13.14
18 Nov 2014	Barren	MC	MC_B6_B	55	41	60	61	100	13	0.80	6.70	7.37	21.37	5.13
	Kelp	LB	LB_K6_K	54	42	88	85	119	16	1.08	40.04	37.66	93.68	21.19
	Rest.	HMC	HMC_T1_R	55	45	79	76	98	14	0.44	18.76	23.67	121.52	20.73
	Rest.	UAC	UAC_J1_R	54	48	78	75	98	12	2.54	27.57	28.18	70.50	16.19



Appendix Fig. A1. The relationship between gonad weight (g) and test diameter size (mm) of *M. franciscanus* collected from April-November 2014 for urchin barrens, kelp reference sites, and restoration sites. Collection date is designated by color.



Appendix Fig. A2. The relationship between gonad weight (g) and test diameter size (mm) of *M. franciscanus* collected from April-November 2014, distributed by the site type (urchin barren: red; kelp reference: green; Honeymoon Cove restoration: light blue; Underwater Arch Cove restoration (dark blue). 95% bootstrapped confidence intervals are displayed for each curve corresponding to the site type. Parameter statistics for estimating curves are in Appendix Table 1.

Developing Growth Promotion Strategies for *Cressa truxillensis* to Improve Success of Restoration Activities

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Abstract.—*Cressa truxillensis*, commonly known as alkali weed, is native to western North America and is used in revegetation projects in saline or alkaline soils at locations such as the Ballona Wetlands Ecological Reserve. This research aimed to (i) determine methods to improve *C. truxillensis* seed germination, (ii) characterize the impact salt has on seed germination and growth, and (iii) identify and characterize bacterial seed endophytes and their potential as plant growth promoting bacteria (PGPB). Results showed that seed scarification, either through mechanical or chemical methods, substantially improved seed germination rates. The presence of salt at 300 mM NaCl delayed germination, and both 150 mM and 300 mM NaCl decreased seedling size. Two different strains of *Paenibacillus peoriae* were found to reside within *C. truxillensis* seeds collected from the Ballona Wetlands. Although neither strain alleviated the salt sensitivity displayed by *C. truxillensis*, both strains showed tolerance to heavy metals, salt, and showed additional properties suggestive that they may function as PGPB. Methods used in this study can serve as guidelines for preparation of seed of *C. truxillensis* prior to seeding in appropriate habitats throughout the species' range.

Wetlands provide important ecosystem services such as supporting biodiversity, filtering water, sequestering carbon, and protecting against storm surge (Zedler and Kercher 2005; Duarte et al. 2013; Benson et al. 2019). Anthropogenic impacts have resulted in a global loss of approximately half of the world's wetlands (Zedler and Kercher 2005). Wetland losses in California have exceeded 90% in the last 200 years, thus creating a continued need for wetland research, conservation, and restoration or enhancement to prevent and slow further loss (Allen and Feddema 1996). Wetlands have increasingly faced threats by invasive species, anthropogenic impacts, and changes in the climate; however, restoration by revegetation with native plants provides one possible solution to returning wetland ecosystem services. Revegetation approaches include transplantation of plants or rhizomes, planting plugs, and direct seeding (Godefroid et al. 2011; Kettenring and Tarsa 2020). Understanding the optimal conditions to promote seed germination and plant establishment of target species is of substantial importance to wetland restoration and management.

A seed-based approach to vegetative restoration has the advantage of being less expensive and logistically less challenging to implement (Kettenring and Tarsa 2020). However, there is a range of soil conditions and hydrology that are likely to affect *in situ* field germination (e.g., nutrient availability, soil grain size and porosity, invasive species presence, etc.), and the downside is that many plant species exhibit extremely low seed germination

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rates, especially due to abiotic stresses such as salt or the presence of a hard seed coat that results in dormancy and prevents imbibition (Almansouri et al. 2001; Baskin and Baskin 2014, 2020). To break dormancy, scarification can break the seed coat and allow a seed to imbibe and eventually germinate (Baskin and Baskin 2014). A better understanding of effective scarification techniques for individual species that are targets of restoration projects is essential for the success of a seed-based approach.

A plant's tolerance to abiotic stress can also be manipulated, with numerous examples of plant growth promoting bacteria (PGPB) forming beneficial relationships with host plants (Cassán et al. 2009; Enebe and Babalola 2018). PGPB can reside within the seed, as well as on and inside plant roots, promoting plant growth and resilience via biochemical properties, resistance to disease, and acting as a buffer to abiotic stressors (Eida et al. 2018). Determining optimal seed germination to account for these inhibitory factors can be important for seeding plants during restoration, leading to a higher percentage of germinated seeds. With a growing need for restoration and improving seeding success of suitable plants for revegetation, this study aimed to inform effective growth strategies of the California native wetland plant, alkali weed, *Cressa truxillensis*.

Cressa truxillensis, commonly known as alkali weed, is a facultative wetland plant native to the western United States and commonly found in many southern California wetlands (Lichvar 2012). *Cressa truxillensis* is known to have a late spring bloom period with hallmark characteristics including a high salinity tolerance and high calcium carbonate tolerance (Jepson and Hickman 1993). Studies on *C. cretica*, another species in the genus, found that the plant could grow in soils with an upper range of 22% calcium carbonate and saline concentrations up to 400 mM (Kabir et al. 2010; Sivasankaramoorthy et al. 2011). Although often found in wetlands, the plant can also thrive as a native in many arid and alkaline habitats, making it an important focus for the conservation or enhancement of multiple habitat types. The *Cressa* genus has very low germination rates in its naturally occurring habitats, which has been attributed to a hard seed coat and suboptimal environmental conditions (Etemadi et al. 2020). This research project aimed to inform the current state of knowledge regarding the use of this species in revegetation projects by investigating *C. truxillensis* scarification techniques and through characterization of its microbial endophytes.

Materials and Methods

Different scarification methods were applied to seeds of *C. truxillensis* to determine optimal conditions to increase the number of seeds that imbibe and germinate. Seeds for the scarification experiment were obtained from S&S Seeds (www.ssseeds.com). *C. truxillensis* seeds are ovular, brown, and 2-4 mm in length (Austin 1998). Four approaches were taken to scarify *C. truxillensis* seeds, including three mechanical and one chemical approach (Table 1). Each treatment had five replicate batches of 20 seeds. Chemical scarification was performed by immersing seeds in 0.5 mL of concentrated sulfuric acid in a centrifuge tube for 45 min. The seeds were then rinsed with sterile distilled water five times to remove all traces of the acid. The three methods of mechanical scarification were scratching individual seeds against sandpaper, rubbing seeds in a batch between sandpaper, and nicking the seed coat with a razor blade. For scarification of individual seeds by sandpaper, each seed was scratched across 220-grit sandpaper for 2.5 cm. For the batch treatment, seeds were randomly dispersed between two pieces of 220-grit sandpaper, a book placed on top to apply even pressure, and a circular motion applied to rub the seeds between the sheets

Table 1. Methods for scarification of *C. truxillensis* seeds

Scarification Type	Chemical	Mechanical		
	Acid	Individual	Batch	Razor Blade
Description	Seeds immersed for 45 min in sulfuric acid (Etemadi et al. 2020).	Seeds individually scratched on sandpaper (Hassen et al. 2005).	Seeds sandwiched between sandpaper, scratched (Hassen et al. 2005).	Seed coat nicked (Mackay et al. 1996).

of sandpaper for 10 passes. To scarify seeds with a razor blade, each seed was nicked once to break the surrounding seed coat. Batches of untreated seeds were placed in petri dishes to serve as controls. For all treatments and the controls, seeds were placed in autoclaved petri dishes containing filter paper saturated with 7 mL of sterile deionized water. The petri dishes with seeds were wrapped in parafilm to prevent evaporation and then placed in the dark at 24°C. The number of germinated seeds within each dish was counted after seven days.

Bacteria associated with *C. truxillensis* were identified, as many plant-associated bacteria have plant-growth promoting properties. *C. truxillensis* seeds were collected from the ground, within *C. truxillensis* and *Salicornia pacifica* plant communities in the non-tidal high salt marsh and transition habitats of the western area B marsh at the Ballona Wetlands Ecological Reserve (Los Angeles, CA, 33.963025°, -118.447015°). No more than 10% of available seed was collected from within individual polygons that were identified throughout the appropriate habitat areas, ensuring that no seed banks were impacted during the collection. A total of 150 seeds were collected from the Ballona Reserve. Seeds were scarified and surface sterilized with 95% ethanol for five minutes, followed by 10% bleach for 10 min, and then rinsed with five washes of sterile deionized water. The water from each wash step was plated onto Tryptone Yeast extract agar (TY; 5 g/L tryptone, 3 g/L yeast extract, 0.0662 g/L CaCl₂·2H₂O, 15 g/L agar) and incubated at 30°C to rule out contamination by a lack of microbial growth on the media. Following sterilization, seeds were crushed individually in 5 mL of sterile saline (0.9% NaCl) using a sterile mortar and pestle. Dilutions of the bacterial suspensions were plated onto TY agar and incubated at 30°C (Siddikee et al. 2010). Dilutions were made so that individual colonies would be easily identified and isolated when grown on TY agar. Two individual bacterial strains, C15 and C3G, were selected to represent the different colony morphologies seen on Yeast extract Mannitol agar (YM; 0.5 g/L yeast extract, 10 g/L mannitol, 0.5 g/L KH₂PO₄, 0.5 g/L K₂HPO₄, 0.2 g/L MgSO₄·7H₂O, 0.2g/L NaCl, 15 g/L agar). A simple stain with safranin and a spore stain were performed on each strain and observed by brightfield microscopy (Zeiss Axioscope 5).

The bacterial strains were characterized for biochemical properties associated with plant growth promotion and their ability to tolerate salt and heavy metal stress. Strains were tested for nitrogen-fixation by growth on nitrogen-free Jensen’s media (Jensen 1942) after 7 d. Cellulase activity was tested by growing the strains on Carboxymethyl Cellulose (CMC) agar (Kasana et al. 2008), flooding the plates with Gram’s iodine, and checking for the presence of clear halos around the streaked bacteria. The production of exopolysaccharide was determined by growing bacteria on YM agar and visually identifying the presence of a mucoid colony morphology. Phosphate solubilization was tested by visually

identifying the presence of clear halos around bacteria on Pikovskaya's agar (Pikovskaya et al. 1948) after 7 d of growth. Auxin production was determined by culturing bacteria in TY supplemented with 1 mg/ml tryptophan and adding Salkowski reagent (Ehmann 1977; Gordon and Weber 1951) after growth. The presence of red coloration indicated the production of indole acetic acid (IAA). For analysis of bacterial tolerance to abiotic stressors, bacteria were streaked on TY agar that had been supplemented with zinc (50, 100, 200, 400, 750, or 1000 μM ZnSO_4), cadmium (50, 100, or 200 μM CdCl_2) or salt (1%, 2%, 3%, 4%, or 5% NaCl), incubated for seven days at 30°C, and growth compared to that seen on TY. All assays were done in triplicate.

To identify bacterial strains C3G and C15, DNA was obtained from each strain using a Chelex extraction (Walsh et al. 2013). The DNA was used to amplify the 16S rRNA gene by the polymerase chain reaction using universal primers 27F and 1492R (Weisburg et al. 1991). The PCR products were sequenced, and the sequences were compared to that of bacterial type strains using tools of the Ribosomal Database Project (Cole et al. 2014) and NCBI BLAST (Altschul et al. 1990). Closely related sequences were retrieved from the GenBank database. A phylogenetic tree of the 16S rRNA gene was generated using MEGAX (Kumar et al. 2018; Stecher et al. 2020) and the maximum-likelihood algorithm. Bootstrap analysis was done for statistical support using 1000 re-samplings with gamma distribution (G) and the Tamura 3-parameter (T92) model (Tamura 1992). The following new sequences were deposited in the GenBank database: 16S rDNA of *Paenibacillus* sp. strain C15 (OK178930) and *Paenibacillus* sp. strain C3G (OK178931).

For the seed germination assays including salt stress and bacterial inoculation, *C. truxillensis* seeds were scarified and surface sterilized as described above. Bacterial strains C3G and C15 were grown overnight in TY media, centrifuged and the bacterial pellet washed once with 10 mM MgSO_4 to remove any residual TY, and then centrifuged again and the bacteria resuspended in 10 mM MgSO_4 to an absorbance at 600 nm of 0.1 (Montañez et al. 2012). *C. truxillensis* seeds were incubated for 1 hr in the bacterial suspension or 10 mM MgSO_4 as a control. The 35 seeds were transferred in triplicate to a petri-dishes containing 0.8% agar (Sigma A1296) with either 0, 150, or 300 mM NaCl. The number of seeds germinated were counted after 7, 14, and 21 days. Wet weight of germinated seedlings was taken at 21 d.

Results

Cressa truxillensis control seeds exhibited a low average percentage of germination when seeds were not scarified ($5.8\% \pm 3.4\%$) (Fig. 1). Chemical scarification in concentrated sulfuric acid for 45 min resulted in the greatest average germination ($54\% \pm 6.1\%$), substantially higher than the unscarified control, although mechanical scarification of individual seeds via sandpaper ($34\% \pm 9.8\%$) and nicking with a razor blade ($29\% \pm 3.4\%$) also improved average germination (Fig. 1). However, batch scarification did not show substantial improvement in the percentage of germination ($11\% \pm 6.1\%$) as compared to the control treatments.

Results indicated that the low salinity treatment (150 mM NaCl) had no impact on percent seed germination of *C. truxillensis* at 7 days ($61\% \pm 1.2\%$) as it was comparable to the control ($59\% \pm 5.1\%$) (Fig. 2). However, the high salinity treatment (300 mM NaCl) caused a reduction in germination ($8\% \pm 0.7\%$) (Fig. 2). By 21 d the percentage of germinated seeds did not vary between saline treatments (Fig. 2). Although the final germination percentages were similar, seedlings exposed for 21 d to 150 mM or 300 mM NaCl showed

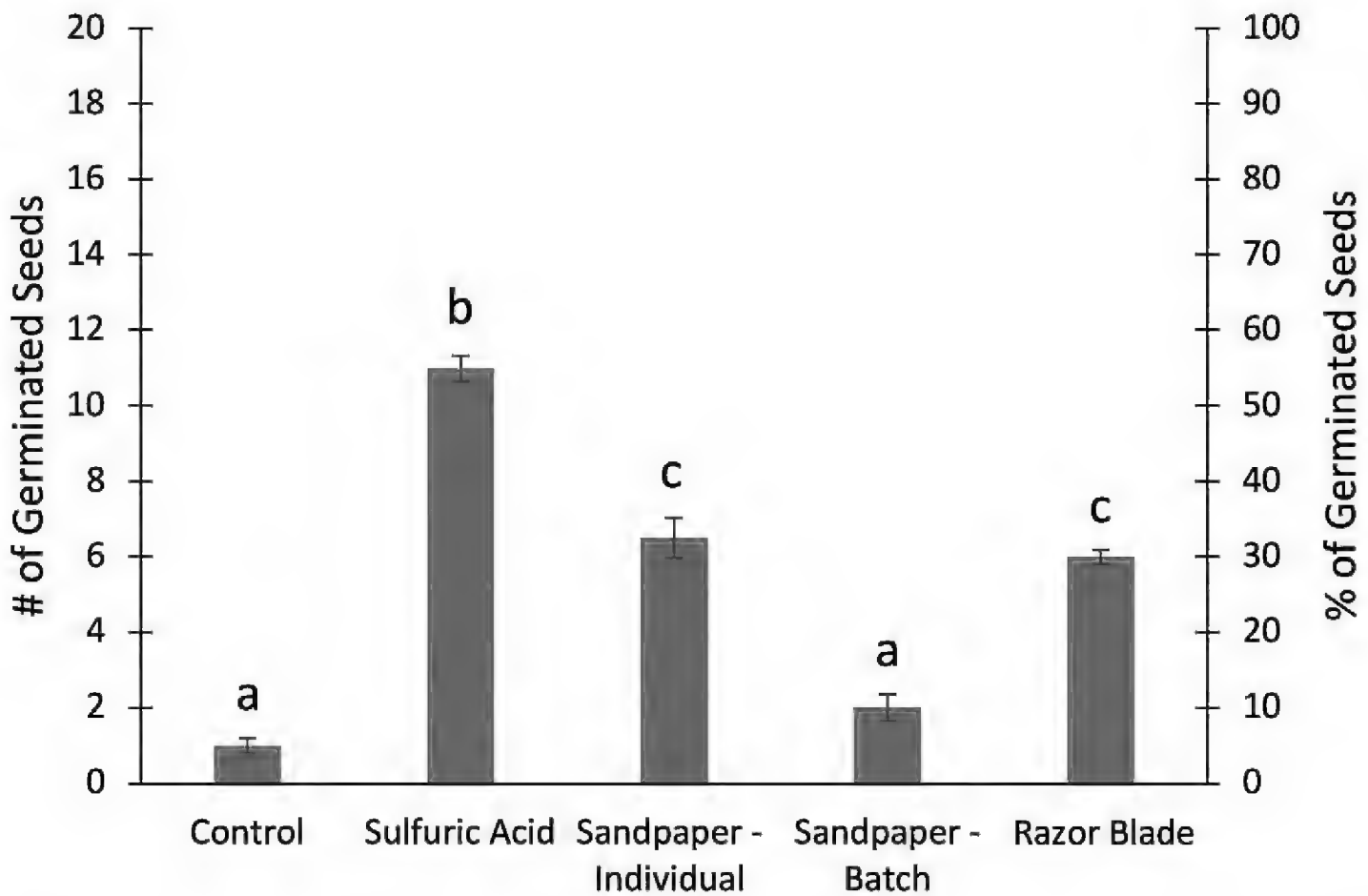


Fig. 1. Effect of four seed scarification treatments on the number of germinated *C. truxillensis* seeds (left Y-axis) and the percentage of germinated *C. truxillensis* seeds (right Y-axis). Values are the mean of five replicates (20 seeds each replicate) and vertical bars indicate standard error. Different letters indicate a statistical difference based on One-Way ANOVA with post-hoc Tukey ($p < 0.05$).

a reduction in growth compared to unexposed seedlings (Fig. 3A and 3B). In addition, the higher the concentration of salt, the greater the reduction in seedling size and mass (Fig. 3A and 3B).

Bacteria were isolated from within *C. truxillensis* seeds that had been collected from the Ballona Wetlands. Although bacterial colonies looked similar on TY, when transferred to YM agar they showed two distinct colony morphologies. Strains C15 and C3G were chosen as representatives of each colony type (Fig. 4A and 4B). Most notably, the texture was a differentiating characteristic between strains C3G and C15; on YM agar, strain C15 had a mucoid appearance indicating the presence of exopolysaccharide while strain C3G did not (Fig. 4B). Microscopy of stained bacteria showed that both strains looked similar and were bacillus in shape (Fig. 4C and 4D). In addition, both strains were found to produce endospores (Table 2). The 16S rRNA gene sequences of strains C3G and C15 were 100% identical to each other and identified them as species of *Paenibacillus*, with 99.71% (1393/1397 n.t.) identity to and phylogenetically grouping with *P. peoriae* DSM8320 (Fig. 5).

Table 2. Biochemical characteristics of endophytic bacteria of <i>C. truxillensis</i> seeds.								
Strain	Tolerance to abiotic stress			PGPB properties				
	ZnSO ₄	CdCl ₂	NaCl (%)	N- fixation	Cellulase	IAA	Phosphate	Endospore
C3G	400 μM	-	4%	+	+	+	+	+
C15	1 mM	50 μM	4%	+	+	+	+	+

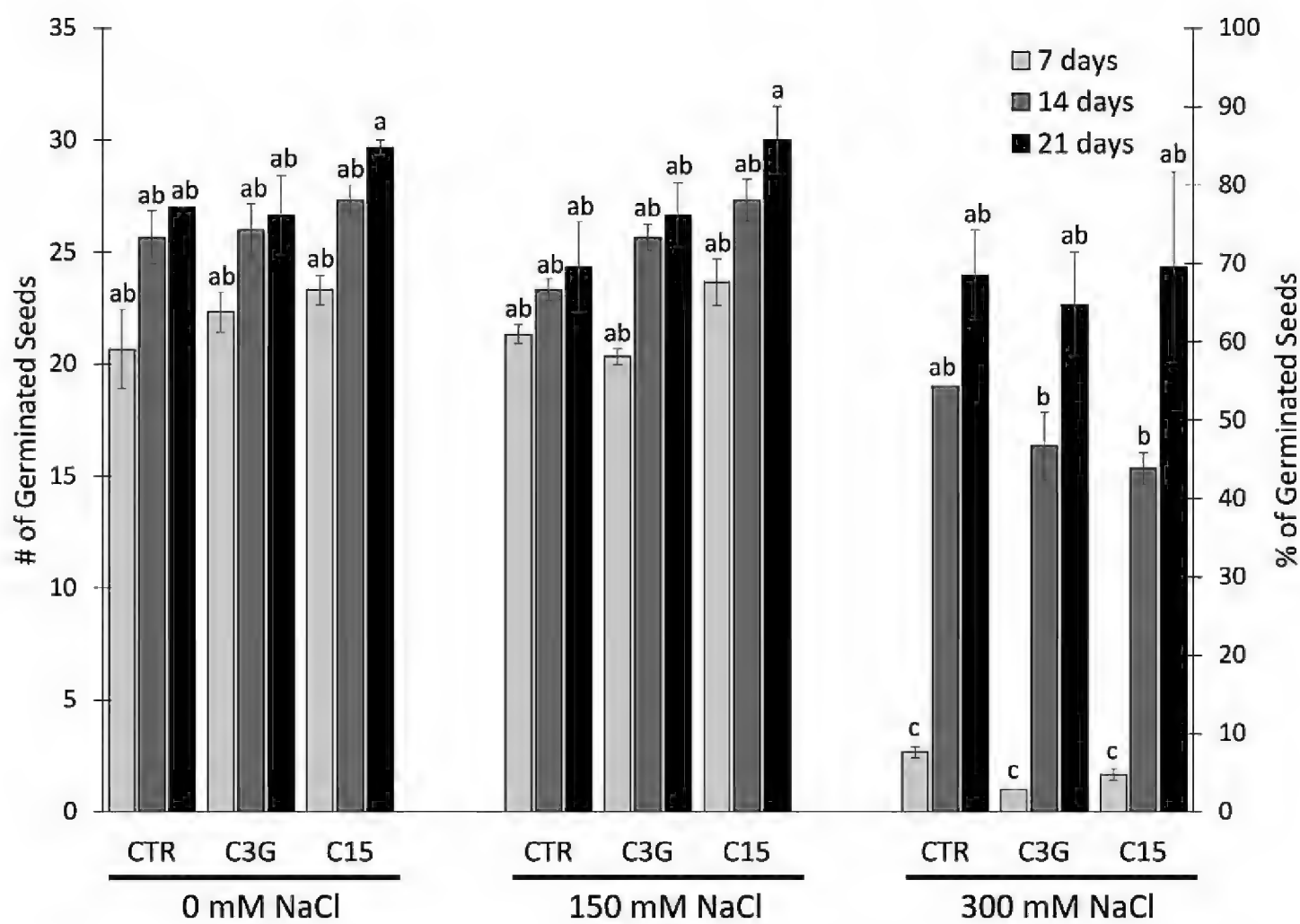


Fig. 2. Effect of NaCl and bacterial inoculation on the number of germinated *C. truxillensis* seeds (left Y-axis) and the percentage of germinated *C. truxillensis* seeds (right Y-axis) over time. Values are the mean of three replicates (35 seeds each replicate) and vertical bars indicate standard error. Different letters indicate a statistical difference based on One-Way ANOVA with post-hoc Tukey ($p < 0.05$). CTR indicates uninoculated control treatments.

Strains C15 and C3G were tested for biochemical properties associated with plant growth-promoting bacteria. Both strains grew on nitrogen-free media (Table 2), indicating that they were able to fix nitrogen, similar to many previously characterized *Paenibacillus* strains (von der Weid et al. 2002). In addition, both strains had cellulase activity, produced auxin, and solubilized phosphate (Table 2). Strains C15 and C3G were tested for growth at varying concentrations of zinc, cadmium, and sodium chloride in order to look at the impact of various abiotic stressors. C3G grew with up to 400 μM ZnSO_4 , however C15 grew even in the presence of 1 mM ZnSO_4 (Table 2). The strains also showed varying tolerance to cadmium. C3G was sensitive to cadmium, but C15 grew in up to 50 μM CdCl_2 (Table 2), highlighting the higher tolerance C15 has to heavy metals. Both strains behaved similarly to salt stress and could grow in as much as 4% NaCl but were inhibited at 5% NaCl (Table 2). When *C. truxillensis* seeds were inoculated with either strain of bacterium, no difference was seen in the percentage of seeds that germinated, whether or not salt was present (150 and 300 mM) (Fig. 2). However, in the absence of salt, inoculation with C3G resulted in a slight increase in seedling wet weight (Fig. 3B).

Discussion

The seed coat can be a major barrier to germination for many plants. How to overcome this seed coat-imposed dormancy, or “hardseededness”, and thus increase the seed’s permeability to water, has been the basis of numerous studies (Tadros et al. 2011; Mousavi

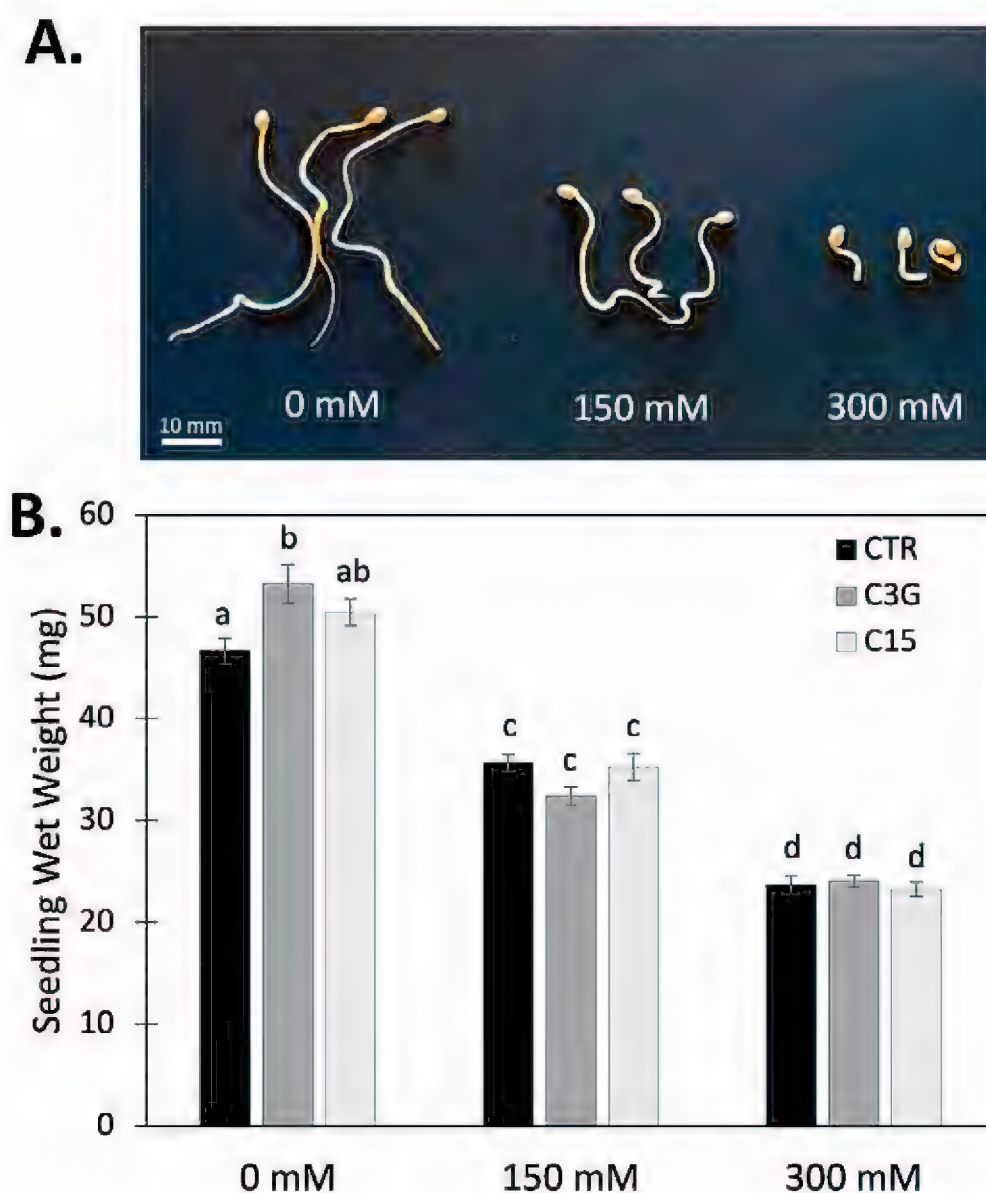


Fig. 3. Effect of NaCl and bacterial inoculation on *C. truxillensis* seedlings. A) Seedlings after 21 d of growth with 0, 150, or 300 mM NaCl. B) Average wet weight of seedlings after 21 d of growth on different concentrations of NaCl. Data represents the mean seedling wet weight from 30 seedlings from three independent replicates, and vertical bars indicate standard error. Different letters indicate a statistical difference based on One-Way ANOVA with post-hoc Tukey ($p < 0.05$).

et al. 2011). Typical scarification strategies include hot water treatment, chemical scarification with acid, or mechanical scarification to nick the seed coat (Rusdy 2017). Though differing in method, chemical and mechanical scarification work to obtain similar results of breaking the seed coat. Mechanical scarification may require more labor on fewer seeds via physical removal or permeation of the barrier, while chemical scarification is effective for larger batches and uses concentrated acid to dissolve part of the seed coat (Majd et al. 2013). The use of sulphuric acid to overcome germination difficulties in seeds has been well-established in the literature as a highly effective scarification technique (Kheloufi et al. 2017). Congruent with studies of *C. cretica*, which found that naturally occurring seeds have very low germination rates (Etemadi et al. 2020), the non-scarified control *C. truxillensis* seeds in this study showed a low percentage of germination. This suggests that restoration or revegetation projects seeding with non-scarified seeds may experience low initial germination rates due to seed coat-imposed dormancy, which would delay bringing back native plant cover to an area. An experimental study at the University of Wisconsin comparing hand seeding and transplantation as restoration techniques found hand seeding to be more successful in producing greater diversity and growth of

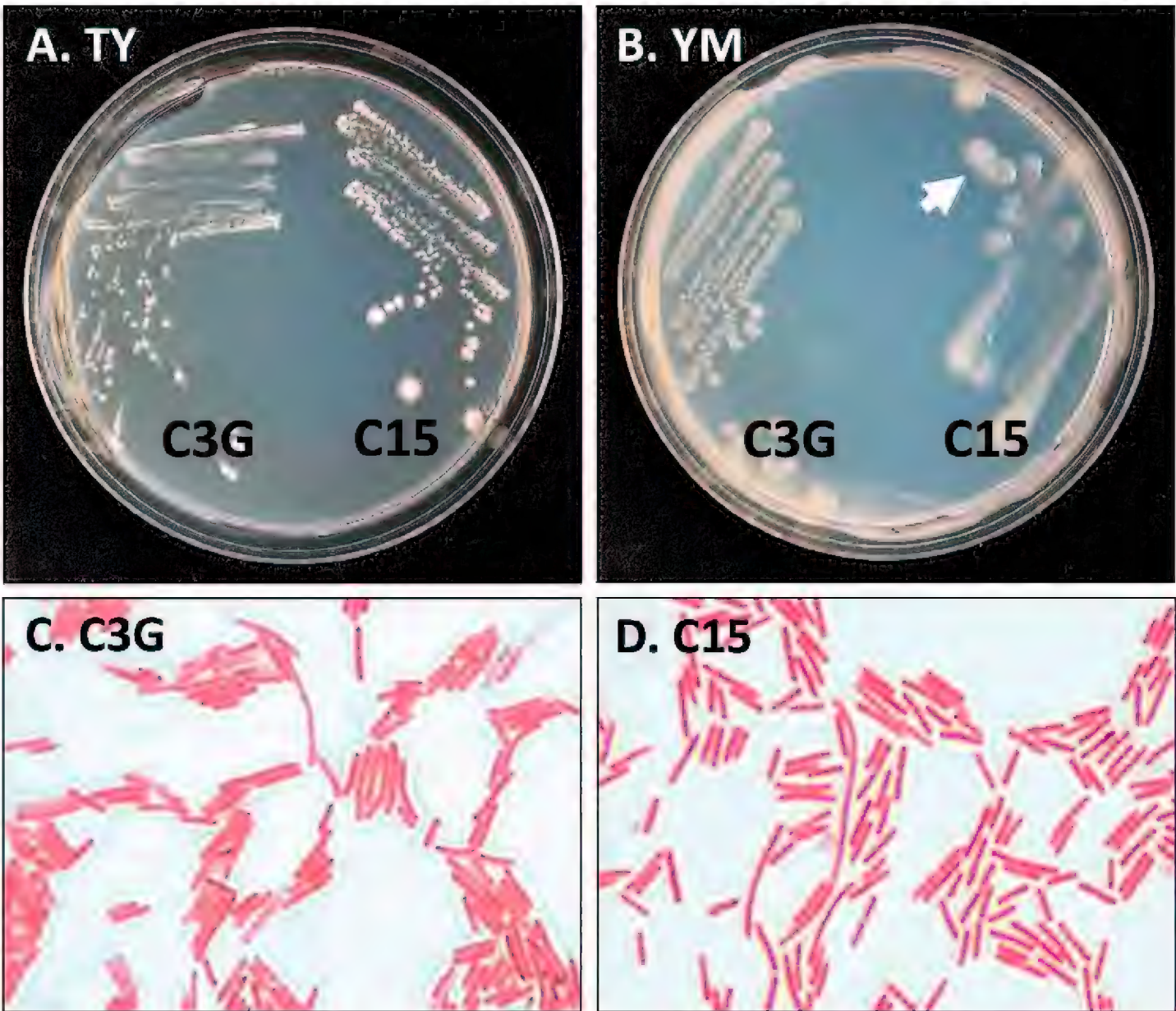


Fig. 4. Colony and cellular morphology of bacterial isolates C3G and C15. Bacterial growth on (A) TY and (B) YM agar. Cellular morphology after staining with safranin of (C) C3G and (D) C15. Arrow points to mucoid colonies of C15.

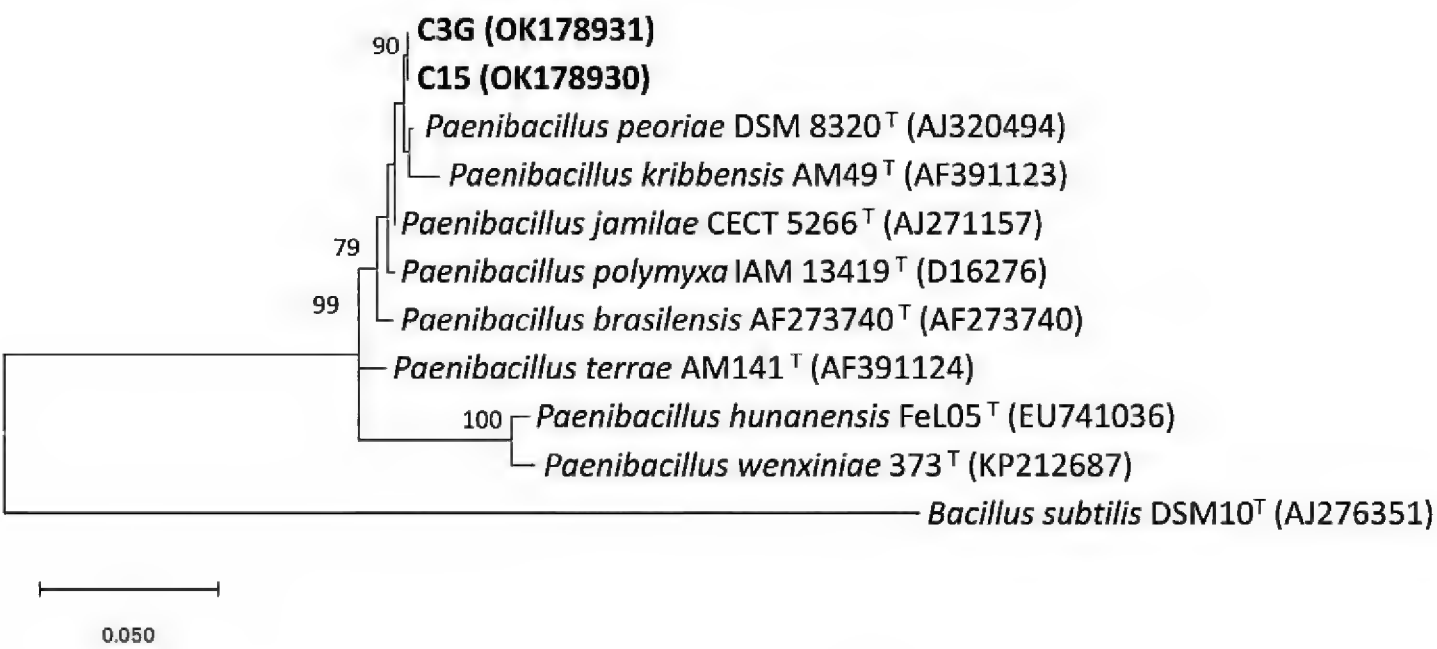


Fig. 5. Maximum-likelihood phylogenetic tree of the 16S rRNA gene sequences from *C. truxillensis* endophytic strains, C15 and C3G, and closely related *Paenibacillus* type strains. Genbank accession numbers are in parentheses. Bootstrap values greater than 70% are shown in the nodes. *Bacillus subtilis* was used as the outgroup.

native plants in addition to greater cost and time effectiveness; however, they noted that an extended dormant period for unscarified seeds may present problems with the regrowth of non-native plants (Weiher et al. 2003). Results from this study indicated both mechanical and chemical scarification methods improved *C. truxillensis* seed germination, showing that scarification is a useful strategy to overcome dormancy. The sulfuric acid scarification technique, which was also effective in scarifying *C. cretica* (Etemadi et al. 2020), substantially improved germination rates of *C. truxillensis* compared to other treatments. Additionally, the sulfuric acid scarification method allowed for many seeds to be scarified at the same time in a batch, meaning this technique could be a feasible option for preparing large quantities of seeds prior to seeding for restoration or revegetation activities, particularly because of the reduced time and effort. If the percentage of seeds that are germinating at each restoration site can be increased using pre-scarified seeds, restoration projects may be able to see more rapid success in reaching native plant cover objectives.

Bacterial endophytes reside intracellularly or intercellularly within the plant, often acting as beneficial PGPB and helping the plant survive abiotic stress (Rashid et al. 2012; Egamberdieva et al. 2017). An increasing number of studies have shown bacteria residing as endophytes in seeds as well (Li et al. 2019; Khalaf and Raizada 2016), with these bacteria likely derived from the parent plant and contributing to increased fitness and survival of the seed in its environment (Li et al. 2019; Mastretta et al. 2009; Zhang et al. 2010). *Paenibacillus* sp. are known to be PGPB for numerous plant species and have been found residing as seed and root endophytes (Eida et al. 2018; Khalaf and Raizada 2016). *Paenibacillus* has been studied in *Arabidopsis thaliana* and found to protect the plant from abiotic stressors and plant pathogens (Hong et al. 2016). Further studies have tagged the bacteria with green fluorescent protein and found that the bacteria form biofilms and colonize root tips of plants (Timmusk et al. 2005). The *C. truxillensis* bacterial strains isolated in this study were identical to each other in 16S rRNA gene sequence and were most similar to *Paenibacillus peoriae*. Although the strains showed variation in exopolysaccharide production and tolerance to cadmium, they both showed plant growth promoting traits such as nitrogen fixation, cellulase production, phosphate solubilization, and auxin production. Consistent with this, after inoculation of *C. truxillensis* seeds, one of the strains resulted in an enhancement of seedling mass. In addition, *Paenibacillus* produce endospores. Bacterial endospores are a dormant form of bacteria that can withstand harsh chemical and thermal environments and allow the bacteria to survive stressful conditions, thus making them ideal for inoculant formulations. If these *Paenibacillus* strains were to be used for plant growth promotion in the field, their endospores could be explored as a form of inoculant as this could increase inoculant shelf life due to the ability of endospores to survive variable conditions (Kim et al. 2010).

Studies have shown that plant associated bacteria, including strains of *Paenibacillus*, can help plants better cope with heavy metal stress (Kumari and Thakur 2018; Sukweenadhi et al. 2018; Eida et al. 2020). The *Paenibacillus* strains from this study showed tolerance to zinc and cadmium. Previous studies in the Ballona Reserve showed that zinc and cadmium concentrations are relatively high, presumably due to urban runoff and other anthropogenic impacts (Johnston et al. 2012). Thus, it is not surprising that bacterial strains isolated from the Ballona Wetlands have increased tolerance to these heavy metals. A study of *C. truxillensis* at a salt marsh in Patagonia, Argentina, found that *C. truxillensis* was sensitive to heavy metals, with morphology of the leaves altered depending on concentrations of pollutants such as zinc (Pollicelli et al. 2018).

Halophytes, which include *C. truxillensis*, are plants that can tolerate NaCl levels greater than 200 mM (1.17%). This is consistent with the study findings for *C. truxillensis*, which could germinate with at least 300 mM NaCl. This finding, as well as the decreased seedling growth seen with increasing salt concentrations, is analogous to what has been reported for *C. cretica* (Etemadi et al. 2020). Recent studies suggest that plant-associated microbes can play a key role in the ability of halophytes to grow in high salinity (Etesami and Beattie 2018) but may also help non-halophytes tolerate salt stress. *Paenibacillus* sp. JZ16, isolated from inside the roots of the halophyte *Zygophyllum simplex*, was found to promote salinity tolerance of *Arabidopsis* (Eida et al. 2018). The *Paenibacillus* strains from this study grew in both the absence of salt as well as in up to 4% NaCl. Both strains also produced auxin, a trait frequently associated with salt tolerant PGPB (Dodd et al. 2010). It is thought that the altered hormonal signaling, which can influence lateral root development (Yang et al. 2009), contributes to the ability of these bacteria to increase the plant's fitness in highly saline environments (Siddique et al. 2010; Tiwari et al. 2011). Other studies have found that exopolysaccharide may allow bacteria to promote plant growth under saline stress (Abbas et al. 2019). Since *C. truxillensis* is known to grow in saline soils, inoculation with an exopolysaccharide producing strain may allow for optimized plant growth in high saline conditions. However, although one of the *Paenibacillus* strains in this study showed some growth promotion of seedlings in the absence of salt, neither strain, whether or not it produced exopolysaccharide, alleviated the negative impact salt had on seed germination or growth under the laboratory conditions tested. Future studies might look at whether these bacterial strains have a beneficial impact on plant growth with salinity stress using greenhouse and field conditions.

Many studies have focused on the use of PGPB in agriculture; however, few studies have explored applications of the microbial community on restoration and native plant revegetation projects (Ahn et al. 2007). The application and results of this study could inform habitat restoration projects throughout the range of *C. truxillensis*, including at the Ballona Wetlands. Wetland restoration projects that have focused on returning native cover to an area have quickly discovered a vast lack of available peer reviewed literature when it comes to best practices for species-specific cleaning, storage, and breaking dormancy, despite breaking seed dormancy being a widely known requirement for revegetation (Kettenring and Tarsa 2020; Barton et al. 2016). Scarification techniques recommended by this study could be used by practitioners working on habitat restoration projects with the goal of improving native plant cover. Past restoration efforts within the Ballona Wetlands have focused on reducing anthropogenic uses and removal of invasive plants within the area (Johnston et al. 2021); however, the findings from this study could allow for a unique opportunity to combine knowledge of revegetation techniques with the microbial community for successful revegetation with *C. truxillensis*. Application of these findings at a restoration project would allow for a greater knowledge base around the use of PGPB for revegetation, as well as development of best practices for germinating wild seeds.

Utilizing the findings from this study, a few key recommendations can be made for future revegetation and restoration projects. Regarding improving germination, sulfuric acid scarification should be used on seeds prior to deployment: this method resulted in the greatest average percent germination and would also allow for large batches of seeds to be scarified together, reducing energy and time. Seeds can be inoculated with PGPB's prior to seeding, such as the ones identified in this study, to increase plants' protections against heavy metals and provide biochemical advantages. Finally, considerations should be taken to deploy seeds in habitats that include some freshwater hydrology, especially in salt marsh

soils. Findings suggested that high salinity had a delaying effect on germination of *C. truxillensis* seeds, and freshwater sources may ameliorate this delay from salt stress to provide maximum germination.

Future directions with these findings will aim to test scarification methods and microbial applications *in situ* for applications within restoration projects. Scarification techniques from this study should also be explored for other native plants commonly seeded in restoration and revegetation projects, including rare species. To further contribute to the available knowledge informing habitat restorations, pre-scarified seeds could be dispersed within an area to measure the success and feasibility of scarification methods in varying field conditions. Based on findings in the Pollicelli et al. (2018) study that *C. truxillensis* has sensitivity to heavy metals, future studies might look at whether the *Paenibacillus* strains from this study are able to increase the tolerance of *C. truxillensis* to certain heavy metals. The endophytic nature and presence of plant growth promoting traits of the two strains isolated in this study suggest they are PGPB, although the strains did not alleviate the impact of salt stress under the conditions tested in this study. Future studies can further assess the conditions in which these strains might promote plant growth and assist *C. truxillensis* withstand abiotic stress, and if this would be helpful when planting seeds collected from other locations that might not carry the same microbes. Formulating inoculants for the plants and measuring growth could give definitive answers on how well each strain is able to promote plant growth and other characteristics that may be advantageous for future restoration projects.

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Knowledge Gaps and Research Priorities in Living Shorelines Science: Insights from Stakeholder Interviews Throughout the U.S. Pacific Coast

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Abstract.—Living shorelines provide a nature-based strategy for coastal restoration with ample opportunity for community engagement and collaboration with interdisciplinary stakeholders. While their implementation has increased over the past few decades, restoration via this technique is limited by several factors including a lack of data sharing among projects and geographical regions, a shortage of long-term monitoring to demonstrate efficacy at meeting project goals, and a need for greater interdisciplinary communication moving forward. In this study, we reviewed recent literature from a range of living shorelines studies throughout the United States and conducted interviews with nature-based coastal restoration practitioners primarily from the U.S. west coast. The insight from these stakeholder interviews allowed us to identify major knowledge gaps about living shorelines and establish priorities for future research and funding, including: (1) funding demonstration projects in their early research stages, (2) supporting projects and trainings for engineers utilizing nature-based infrastructure, (3) conducting long-term monitoring of both ecological and structural properties, (4) communicating findings, importance, and project visualizations to stakeholders within and between communities, and (5) advancing the causes of environmental justice and equity. By reviewing recent literature and engaging with living shoreline practitioners to gather their experiences and suggestions, we have increased understanding of how living shoreline restoration can be more effectively planned, constructed, and monitored at scale, in varied locations and using a range of techniques.

Living shorelines restoration is an increasingly important method of enhancing coastal resilience in the face of changing climate and rising sea levels, as it provides nature-based alternatives to shoreline armoring while maintaining biodiversity (Bilkovic et al. 2016; Gittman et al. 2016b; Smith et al. 2020). Coastal restoration using natural infrastructure furthermore presents opportunities for community engagement and collaboration among diverse groups of stakeholders (Bragg et al. 2021; Molino et al. 2020; Smith et al. 2020). These benefits to local ecosystems and communities demonstrate the value of utilizing living shorelines for coastal resilience on a larger scale. In order to broaden the use of living shorelines, however, several limitations to their widescale implementation must first be addressed.

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First, a consistent definition of living shorelines is lacking; many types of coastal habitat restoration in different geographic locations are encompassed by the term “living shorelines” (Moosavi 2017; O’Donnell 2017; Smith et al. 2020). Furthermore, living shorelines are highly context-dependent, and extending their use to new coastal communities requires careful consideration of regional needs and management concerns. Compilations of case studies varying by landscape setting, energy level, and habitat type are a work in progress, but a more concentrated effort in developing these resources is needed (Beagle et al. 2019; SCC et al. 2010; Judge et al. 2017). The concept of using living shorelines is still relatively new, particularly on the Pacific coast of the United States (Bilkovic et al. 2016; Boudreau et al. 2018; Smith et al. 2020). Restoration designs on the U.S. west coast therefore often draw from East coast case studies and need to be adapted to Pacific shorelines, which are characterized by more open coast area and shoreline hardening (Boudreau et al. 2018; Gittman et al. 2015; Hanak and Moreno 2012). Demonstration projects testing new techniques and evaluating the likelihood of their success, especially along the Pacific coast, are limited (SCC et al. 2010; Russell and Griggs 2012; Saleh and Weinstein 2016). A central goal of our research was to investigate regional differences among approaches to living shorelines, highlighting projects on the Pacific coast through stakeholder interviews to gain a better understanding of the challenges specific to this region.

One particular area that merits attention is discussion with stakeholders about successes, perceptions, and limitations to implementing living shorelines restorations. Several studies have compiled information on different restoration methodologies through discussion with experts and literature review to identify restoration goals and guidelines (Baggett et al. 2015; Fitzsimons et al. 2020; O’Donnell 2017; Smith et al. 2020; Ridlon et al. 2021; Waltham et al. 2021; Zeigler et al. 2021). These studies have highlighted the need for greater discussion and data sharing among coastal restoration practitioners. Some research furthermore included a practitioner interview or survey component (Fitzsimons et al. 2020; Molino et al. 2020; Smith et al. 2020); however, direct interviews with interdisciplinary stakeholders have a very limited representation in living shorelines literature. Through both a literature review and interviews with coastal restoration practitioners from a variety of disciplines and communities, we identify scientific knowledge gaps, priorities for future research and opportunities for community engagement.

Materials and Methods

The first component of our study consisted of a review of relevant literature encompassing diverse perspectives in living shorelines science. The databases Academic Search Complete, ScienceDirect, Web of Science, and JSTOR were used in addition to Google Scholar to find peer-reviewed research articles on living shorelines science, from foundational discussions to recent studies incorporating novel methodologies. Several search terms were used to find literature, given the inconsistencies in living shorelines definitions and the variety of methods used by practitioners. “Living Shorelines,” “Nature-Based Infrastructure,” “Nature-Based Engineering,” “Coastal Resilience,” “Shoreline Hardening,” “Coastal Restoration,” “Coastal Management,” “Oyster Seagrass Restoration,” and “Multi-Habitat Coastal Restoration” were all searched in each database to find relevant literature, with secondary terms added including “stakeholders,” “interviews,” “practitioners,” “community,” and “community engagement.” In addition to the journal articles resulting from these searches, we furthermore reviewed publications from nonprofit organizations (e.g., The Nature Conservancy), state and local government

Table 1. Overview of the distribution, disciplines and affiliations of stakeholders interviewed between March and June of 2020.

Category	Stakeholders interviewed
Geographic region	
Northern California	7 (30%)
Southern California	12 (52%)
Washington State	1 (4%)
Hawaii	1 (4%)
East Coast	1 (4%)
Gulf Coast	1 (4%)
Discipline	
Biologist	6 (26%)
Oceanographer	1 (4%)
Engineer	4 (17%)
Policymaker	2 (9%)
Funding Work	2 (9%)
Geomorphologist	1 (4%)
Coastal Manager	3 (13%)
Affiliation	
Engineering or Consulting Firm	5 (22%)
Federal Agency	1 (4%)
Funding Organization	1 (4%)
Grassroots Initiative	1 (4%)
NGO	2 (9%)
Nonprofit	3 (13%)
Research Institute	1 (4%)
State Agency	3 (13%)
University/Academia	6 (26%)

agencies (e.g., the California State Coastal Conservancy), white papers, proceedings from academic society and local government meetings, and mission statements from funding organizations.

The second component of this study consisted of direct interviews with stakeholders involved in living shorelines restoration efforts, also referred to in this study as practitioners to reflect their active involvement with restoration projects. These living shorelines practitioners were selected with the goal of equally representing different disciplines and organization types along the Pacific coast. Some interviewees based in the East and Gulf coasts were included due to their familiarity with projects in the Pacific, or their specialized knowledge of techniques that could be applied to Pacific coast systems in the future. A list of potential contacts was created throughout the literature review process and was expanded through input solicited from several experts in the field of coastal restoration. Interviewees were selected from nonprofit and grassroots organizations, state and federal agencies, academia, consulting and engineering firms, and funding groups. Biologists, ecologists, oceanographers, engineers, policy specialists, and stakeholder engagement professionals all participated in interviews. Please refer to Table 1 for an overview of the organization types and disciplines represented among interviewees, in addition to their geographic distribution.

Interviewees took part in a phone or video call ranging from 25 mins to 1.5 hrs in length to discuss challenges and opportunities in living shorelines science. The following questions were asked to each practitioner:

1. What have you found to be the biggest challenges or barriers in advancing living shorelines research in your work?
2. What projects and programs do you think are most needed to help advance living shorelines and on-the-ground climate resilience measures?
3. What research topics or innovative restoration methodologies would you be interested in learning more about, both in your community and in other regions? Are there specific novel techniques for increasing coastal climate resilience that you think need more support or attention?
4. What are the needs to help enhance scalability of living shorelines projects beyond individual locations?
5. How is your living shorelines research being used by practitioners and communicated to the public, and how might this be improved upon?
6. Are you seeking any new partnerships or methods of community engagement that would be particularly valuable in accomplishing your project goals?
7. How can communication around advancements in living shorelines science and solutions be enhanced within the scientific community?

A total of 23 living shorelines practitioners participated in interviews between March and June 2020. While 34 practitioners were identified and contacted for interviews based on our literature review and additional input from experts, the 23 interviewees described here represent the subset of practitioners contacted who were responsive to our request and able to participate. Interviews were conducted between March and June 2020. After interviews were completed, individual responses to interview questions were reviewed and common themes were identified. The major challenges and opportunities discussed by practitioners were then compared to those documented in the literature.

Results

Our literature review included a total of 50 sources, incorporating perspectives on living shorelines projects from the East, West, and Gulf coasts of the U.S. (Fig. 1). The sources reviewed included journal articles on small-scale living shorelines projects monitored primarily by academic researchers, as well as case study compilations for larger collaborative efforts between nonprofits and agencies at the State or Federal level. Pacific coast studies were more limited in scope; of the 50 sources reviewed, only 18 (36%) were specific to the Pacific coast. Among Pacific coast studies, projects were clustered in areas with low-energy back bays, including San Francisco Bay and Elkhorn Slough in northern California; Newport Bay and San Diego in southern California; and Puget Sound in Washington State. The geographic distribution of sources highlighted the relative lack of literature and case studies from West coast systems, a result of living restoration techniques being relatively newer to this region. Comparing sources from both regions allowed us to distinguish knowledge gaps unique to the Pacific coast from those that are overarching needs in living shorelines science.

The literature review identified a variety of challenges in living shorelines science, which are summarized in Table 2 and compared with practitioner-identified challenges. First, there is a critical need for quantitative assessments of long-term structural integrity of living shorelines, as well as research into their maintenance requirements and success in adapting to rising sea levels. Another widely cited knowledge gap is a lack of projects directly comparing the effects of “green” or natural versus “gray” or artificial structures. The incorporation of any artificial element into a living shorelines design is controversial

Table 2. Commonly identified challenges and knowledge gaps in living shorelines research and literature, grouped by number of interviews in which each was discussed: (A) ≥ 10; (B) 5 to 9; and (C) < 5.

	Common needs & challenges	Examples & Action needed	Mentions (Stakeholders <i>n</i> = 23)	Mentions (Literature <i>n</i> = 50)
A	Long-Term Monitoring	Need for 10+ years of data on living shorelines durability, adaptation, ecological response, & ecosystem services	13 (57%)	31 (62%)
	Demonstration Projects	Pilot projects in new communities and varied coastal contexts	13 (57%)	16 (32%)
	Engineer Training and Resources	Design guidelines, standardized methods, and training/certification programs in natural infrastructure	13 (57%)	18 (36%)
	Communications Campaigns	Social media outreach, video projects, community environmental education	12 (52%)	14 (28%)
	Green-to-Gray Spectrum	Need for direct comparisons of man-made and natural structures and case studies in high-energy environments	11 (48%)	25 (50%)
	Cross-Disciplinary Networks	Increased communication and data/results sharing between scientists and professionals, policymakers	11 (48%)	29 (58%)
	Success Rate	Need for data on living shorelines' efficacy in achieving long-term goals (i.e., coastal protection, habitat value)	11 (48%)	25 (50%)
	Visualizations	Maps, imaging, drone photos, virtual reality, models, apps; any way to display projects and gain community attention	10 (43%)	3 (6%)
B	Consistent Definitions	Disagreements on what habitat types, coastal contexts, and structural elements constitute living shorelines	9 (39%)	9 (18%)
	West Coast Guidelines & Examples	Regional compilations of shoreline designs appropriate for different settings, expanding on successes and failures	9 (39%)	10 (20%)
	Permit Streamlining	Pilot-scale, streamlined permitting with easily accessible checklists to enable more efficient preparation	8 (35%)	14 (28%)
C	Environmental Justice & Equity	Need for outreach in under-served communities	5 (22%)	10 (20%)
	Dialogue Between Communities	Sharing concerns, stories, goals, successes, challenges	5 (22%)	11 (22%)
	Assessment of Public Perception	Stakeholder outreach (i.e., public meetings, webinars, surveys)	5 (22%)	22 (44%)
	Documentation of Timeline & Cost	Availability of information (material cost, permitting timelines, project maintenance) to streamline future work	4 (17%)	20 (40%)
	Regulatory Agencies	Increased advocacy to demonstrate importance of living shorelines and maintain funding	3 (13%)	20 (40%)
	Building Ground-up Demand	Increasing community support for healthy natural coastlines and extending support beyond scientific community	2 (9%)	9 (18%)



Fig. 1. Map depicting geographical distribution of interviewees and literature sources consulted in this study. Stakeholders interviewed are in red; literature sources are in black.

among living shorelines practitioners (Boudreau et al. 2018; Moosavi 2017). While some advocates of hybrid green-gray approaches are encouraged by the durability of artificial elements in the face of high wave energy, many biologists are concerned that small-scale habitat restoration adjacent to armoring will add little ecological value and do nothing to address the problem of hardened shorelines.¹ Living shorelines themselves, when in the proper coastal setting, have been documented to be more effective in reducing the impacts of storms than armored structures (Bilkovic et al. 2016). However, in open coast, high energy environments, success is limited (Walker et al. 2011). More research into these high-energy coastal contexts, especially during extreme weather events, is needed (Gittman et al. 2015; Hanak and Moreno 2012; Saleh and Weinstein 2016). Detailed risk assessments and hazard modeling are imperative in understanding the durability of living shorelines into the future and assessing the role they will play in reducing flood risk (Aerts et al. 2018; Reguero et al. 2018; Russell and Griggs 2012).

Long-term monitoring of ecosystem services has been highlighted by numerous sources as another essential component to living shorelines success, especially regarding wave attenuation, faunal community response, and carbon sequestration (Benayas et al. 2009; Bilkovic et al. 2016; Davis et al. 2015; Gittman et al. 2016a; Patrick et al. 2016; Simenstad et al. 2006; Zeigler et al. 2018, Ridlon et al. 2021). These long-term data convey the success or failure of nature-based infrastructure at meeting its coastal resilience goals, and are needed to assure coastal property managers, landowners, and policymakers that their investment in natural alternatives to shoreline armoring is worthwhile (Baptist et al. 2019; Sutton-Grier et al. 2018). Next steps include streamlining communica-

¹ Pilkey, O.H., Young, R., Longo, N., Coburn, A., 2012. Rethinking Living Shorelines. Program for the study of developed shorelines. Program for the Study of Developed Shorelines, Western Carolina University, 10 pp. Available from: <http://www.oyster-restoration.org/wp-content/uploads/2012/06/Pilkey-et-al.-Final-LS-White-Paper.pdf>. Accessed 29 January, 2022.

tion between science and management and improving public perception of living shorelines (Bragg et al. 2021; Currin 2019; Smith et al. 2020; Waltham et al. 2021)². Clearer communication of living shoreline benefits to the public using specific examples will establish ground-up demand and lead to the institutional capacity building required for larger-scale implementation.³ In short, a number of logistical and sociological hurdles underlie the major knowledge gaps in living shorelines research, indicating areas where support is most needed.

Many of the major challenges and barriers to living shorelines advancement identified in the literature review corresponded with the findings of the interviews (Table 2). Practitioner responses to interview questions varied to some extent by discipline, but many responses were common or interconnected among interviewees. Fig. 1 indicates the geographical range of interview participants; most practitioners were from northern or southern California (Table 1), reflecting the clustered distribution of living shorelines projects observed in the literature review. The majority of interviewees and research articles consulted both indicated that one of the most pressing challenges is a lack of long-term data evaluating how living shorelines projects are meeting their specific goals, particularly along the Pacific coast given that living shorelines have historically been concentrated in East coast systems. Living shorelines projects are often monitored for a few years past their implementation, generally up to about five years with few exceptions. There is a simultaneous need for more demonstration projects to begin and for monitoring of existing projects to continue, a need that has been consistently identified over the past decades and prompted many suggestions from interviewees. West coast living shorelines studies require further research into project lifespan, standardized timelines and monitoring methods, and effective restoration materials in different coastal settings. A growing network of professionals and a collaborative effort to share data and methods is needed to guide future research and streamline restoration processes in the future. As is further described in the discussion, interviewees identified several approaches to increase community investment in restoration projects for long-term persistence and positive perceptions of new projects.

Interviewees across multiple disciplines and organization types identified a great need for capacity building when it comes to living shorelines restoration, especially professional training programs. Many practitioners mentioned a lack of engineers trained in nature-based infrastructure and familiar with its unique benefits and challenges, a limitation also identified various times within the literature review. For living shorelines to become a more widely used engineering practice, long-term studies of durability, adaptation, and maintenance needs are also essential, especially in high-energy environments. Specific regional design guidelines should be created to distinguish which techniques have succeeded and failed in a given coastal setting. These specifications can inform training and information sessions for interested practitioners and can refine the method selection process when designing future projects.

Improved communication with regulatory agencies was also identified by multiple practitioners and various literature sources as an area where further work is greatly needed.

² Belcher, A., Bezore, R., Covi, M., Yusuf, W., 2019. Living Shorelines: Barriers and Promotion: Accomack County, VA. Old Dominion University. 10pp. Available from: <https://digitalcommons.odu.edu/cgi/viewcontent.cgi?article=1019&context=odurc-presentations>. Accessed 29 January, 2022.

³ Restore America's Estuaries, 2015. Living Shorelines: From Barriers to Opportunities. Arlington, Virginia. 55 pp. Available from: <https://estuaries.org/wp-content/uploads/2019/02/Living-Shorelines-From-Barriers-to-Opportunities.pdf>. Accessed 29 January, 2022.

Policymakers rely on concrete evidence that living shorelines work as intended, and this is not achievable without ample scientific support and effective communication. Maintaining dialogue between science and management will improve regulatory agencies' understanding of living shorelines projects as well as researchers' understanding of permitting requirements. Streamlining the permitting process for future projects will enable new living shorelines projects to get off the ground more efficiently and will make them an accessible strategy for more communities and small nonprofits. Finally, increased community engagement and data sharing is needed to shift public perceptions of living shorelines and promote the concept of natural beaches and their benefits.

To address the challenges listed above, interviewees proposed a wide range of solutions from innovative restoration studies to educational programs and communications campaigns. The most commonly mentioned approaches are outlined in Table 3, as well as the prevalence of these approaches in living shorelines literature. The discussion and recommendation section overviews many of the most commonly referenced suggestions for specific project types, but several one-off suggestions are listed in Table 3 as well. Some of these include unconfined sediment placement from dredge material as a method of sea level rise mitigation, analysis of nature-based infrastructure in groundwater studies, where adaptability is a clear advantage over armoring, and research into natural irrigation techniques for restoration projects during drought periods. Other suggestions were specific to oyster restoration, including the development and use of natural reef balls, the implementation of shell recycling programs and networks, and the creation of higher-relief oyster beds for greater structural stability. Finally, increasing the accommodation space between coastlines and hardened structures to accommodate wave action was identified specifically in one interview as a valuable research area, though this echoed the needs outlined by many interviewees.

Discussion

Both the literature review and stakeholder interview components of this study provided insight into current limitations in living shorelines science, as well as priority areas for future research. Interviewees suggested a variety of strategies, projects, and programs needed to address these challenges. They further identified areas where available funding can be effectively placed to create a scalable impact. These recommendations built upon many of the conclusions outlined within living shorelines literature and centered on the five following topics: supporting innovative demonstration projects, supporting engineer trainings and needs, funding long-term monitoring, investing in communications and media projects, and prioritizing community engagement. The focus areas identified through this analysis provide a guide for future restoration and funding efforts.

Supporting Demonstration Projects with Innovative Methods.—Supporting pilot projects in their early research and development stages was one of the most frequently suggested ways to make a scalable impact in living shorelines research. Lack of funding for initial implementation of new adaptation strategies remains a major barrier, especially in Northern and Central California (Moser et al. 2018). By funding preliminary research in its academic and/or early planning stages, strategically placed funding and community engagement can help demonstration projects get off the ground. With effective communication and documentation throughout the project, this initial support can then be used to leverage public funds on a larger scale (e.g., grants from state and federal agencies) (Fitzsimons et al. 2020). Furthermore, to encourage restoration efforts on

Table 3. Opportunities for supporting innovative restoration techniques and programs identified by interviewees and within literature, grouped by number of interviews in which each was discussed: (A) ≥10; (B) 5-9; (C) 2-5; (D) one-off suggestions within interviews.

Methodologies & Research areas		Examples	Mentions (Stakeholders <i>n</i> = 23)	Mentions (Literature <i>n</i> = 50)
A	Green-gray hybrid structures	Studies directly comparing benefits and drawbacks of nature-based infrastructure, and/or assessing the incorporation of limited man-made materials in high-energy environments	11 (48%)	25 (50%)
	Open coast and offshore studies	Studies assessing offshore vegetation efforts (i.e., kelp and eelgrass restoration) and resulting changes in sediment deposition, especially as shorelines migrate	10 (43%)	6 (12%)
B	Hazard and risk modeling	Predictions for long-term adaptation and durability, addressing rising sea levels and ability to withstand disturbance	7 (30%)	12 (24%)
	High wave energy settings	New approaches to restoration in high-energy environments	7 (30%)	21 (42%)
	Community science	Involving the local community in long-term data collection to both educate and build a collaborative dataset	6 (26%)	9 (18%)
	Identifying future habitat	Projects that plan ahead and identify habitat/land areas that will be needed for managed retreat	6 (26%)	13 (26%)
	Cobble studies	Recommended by engineers: research into the use of cobble as a natural infrastructure material (e.g., durability, movement)	5 (22%)	5 (10%)
	Preparation for public meetings	Providing food, arranging webinars, bringing people together	5 (22%)	3 (6%)
C	Dune revegetation	Recommended by engineers: research into revegetated dunes and how they grow, accumulate sediment, and protect shores over time	4 (17%)	4 (8%)
	Ecosystem service quantification	Studies calculating the economic value of services provided by restoration (water clarity, biodiversity, storm protection)	3 (13%)	13 (26%)
	Extreme weather events	Studies addressing ability to protect against storm surges	3 (13%)	16 (32%)
	Experimental design	Studies with randomized, replicated experimental designs	3 (13%)	10 (20%)
	Shell recycling programs	Building a growing network of local restaurants and businesses as partners, providing shell for oyster restoration	2 (9%)	3 (6%)
	Multiple habitat types	Connecting as many habitats as possible from subtidal to upland	2 (9%)	9 (18%)
D	Dredge sediment monitoring and placement	One USACE program, “Innovative Shore Protection”, once monitored dredge sediments and needs replacement. These sediments could be put to use in sea level rise mitigation efforts.	1 (4%)	6 (12%)

Table 3. Continued

Methodologies & Research areas	Examples	Mentions (Stakeholders <i>n</i> = 23)	Mentions (Literature <i>n</i> = 50)
Community-based subsistence areas	Collaboration between local government and community, i.e., indigenous fish ponds and community subsistence areas in HI	1 (4%)	2 (4%)
Natural reef balls	Alternatives to artificial reef balls used in oyster restoration	1 (4%)	3 (6%)
High-relief oyster beds	Need for taller oyster bed structures given the low relief of Olympia oyster beds; Baycrete may be a helpful material	1 (4%)	2 (4%)
Groundwater-based natural infrastructure	Using nature-based infrastructure in groundwater, where adaptability is important and armoring is undesirable	1 (4%)	3 (6%)
Accommodation space	Projects focused on increasing the amount of space between wave action and hardened structures	1 (4%)	0 (0%)
Stormwater irrigation	Using stormwater to maintain restored habitats, potentially used in a larger-scale project such as a horizontal levee	1 (4%)	1 (2%)
Drought studies	Natural irrigation techniques for drought periods where restored vegetation may begin to die off	1 (4%)	2 (4%)

privately owned lands, economic incentives should be promoted and publicized to attract the attention of private landowners who may otherwise not have interest in, or knowledge of, implementing a living shoreline on their property (Scyphers et al. 2020). The next few paragraphs detail several research areas that should be a priority in coastal restoration based on their innovative methodologies.

First, projects involving risk assessment and evaluating the potential for managed retreat are greatly needed. Many practitioners reiterated the challenge of a highly urbanized and developed Pacific coastline, especially in southern California, which requires restoration approaches that cannot be applied directly from case studies in other regions. Adding habitat value to these hardened coastlines is crucial, as is planning for managed retreat in the face of sea level rise. Interviewees consistently identified risk assessments as a way to increase scalability of living shorelines. Sea level rise modeling and incorporating extreme weather events will inform next steps, including site selection for future pilot projects. It will also aid in identifying “future habitat” where coastal habitat restoration efforts should be focused to transition to higher sea levels; this can range from efforts to convert agricultural land that will be unusable in the future or removing infrastructure currently in place that will not withstand sea level rise. These risk assessments will further aid in developing estimates of cost and shoreline protection value, a key piece of information for policymakers (SCC et al. 2010; Reguero et al. 2018).

Research assessing living shoreline success in high-energy environments is also needed to inform management strategies, specifically restoration site selection, project design, and expectations for structural persistence and maintenance. Living shorelines have generally

been limited to low-energy areas such as back bays in order to introduce nature-based infrastructure in areas with greatest potential for success. The focus must now be extended to regions where wave energy and erosion are high, in order to further demonstrate their efficacy to engineers, policymakers, and other stakeholders involved in coastal management. In these high-energy areas, several methodologies must be considered and directly compared. This is especially true in systems where “gray” infrastructure is more commonly used, readily available, or easy to implement. Considering the use of hybrid “green-gray” structures may be a valuable first step in increasing resilience within highly urbanized communities, while simultaneously increasing visibility of and engagement with living shorelines (Moosavi 2017; Silvertooth et al. 2019). For example, revegetation efforts along a horizontal levee or habitat restoration adjacent to a seawall already in place will increase ecosystem services and present a first step in using natural structures as an alternative or draw attention to removing the armoring itself. Similarly, using a breakwall in conjunction with oyster restoration was found to enhance ecosystem recovery in a high-energy environment in Florida (Safak et al. 2020). These approaches must be considered very carefully, given that many researchers are well aware of the scant ecological value of projects closer to the “gray” end of the spectrum and caution that the habitat restoration is more of a disguise for armoring than an adaptive approach to coastal resilience (Smith et al. 2020)¹.

To better inform engineers, policymakers, and other stakeholders involved in coastal management, studies directly assessing benefits and drawbacks of green versus gray structures in different Pacific coast systems would be highly useful. Beyond establishing pilot projects and monitoring their success, a coordinated effort is needed across the Pacific coast to test green infrastructure in a series of high-energy coastal environments. This type of regional study would provide replicated data to engineers, geomorphologists, and policymakers that can inform which conditions are ideal for fully natural solutions such as dunes and, on the other hand, which conditions may require an understory of rock. It would furthermore help identify instances in which using durable hybrid elements as one component of a living shoreline might offer a better, or transitional solution. For example, Baycrete/ECONcrete (concrete mixed with natural materials such as shell or sand) may present an opportunity to construct high-relief oyster beds, considering the limited bed size of the native Olympia oyster. Projects that entail standardized monitoring metrics over an extended period (10+ years) and clearly communicate their results to scientists across the Pacific coast are a funding priority based on the findings of our interviews and literature review.

In addition to advocating for studies in high-energy environments, many practitioners and particularly engineers expressed that research on the open Pacific coast provides further opportunities for innovation. Most interviewees with this suggestion worked at the interface of science and management at relatively large scales, indicating that these projects are especially important next steps in the big picture of advancing coastal resilience. Some specific examples include restoration of offshore vegetation, including kelp and eelgrass; studying their impacts on sedimentation patterns and wave attenuation will become increasingly important with sea level rise and shoreward migration. Projects linking increased numbers of habitat types from upland to offshore should also be given priority, as they will provide a higher degree of structural diversity, thereby increasing habitat value and wave attenuation (Zeigler et al. 2018). These types of open coast studies are also important in better understanding engineering needs in higher-energy environments

(Walker et al. 2011).⁴ Compiling a coordinated set of data to inform the restoration technique selection under a given set of physical and biological conditions should be a priority to fill this knowledge gap and provide resources for future practitioners.

Finally, demonstration projects with an experimental design, especially a randomized replicated design, are needed to reinforce scientific support behind living shorelines projects (Gellie et al. 2018). Replication along the Pacific coast will establish the settings in which different approaches are most effective as well as their projected likelihood of success. Data sharing among these projects and dialogue between the communities in which they take place should be facilitated. The results of these studies can be combined to contribute to a regional guide outlining different techniques and the best contexts in which to use them.

Supporting Engineer Trainings and Needs.—The low number of engineers working on living shorelines projects was identified in the literature review as a common challenge for restoration efforts across the U.S. (identified in 36% of sources reviewed; see Table 2). A need for more engineer involvement on Pacific coast projects in particular was emphasized by a majority of practitioners interviewed (57%; Table 2). In the context of the interviews, the definition of engineers included geomorphologists with expertise in the structural dynamics of coastal ecosystems, geologists studying shoreline processes, and GIS practitioners assessing structural changes over time. To increase engineer interest and involvement in living shorelines projects, more certified training programs in natural infrastructure are needed as well as targeted recruitment efforts through university interest groups, seminars, and collaboration with interdisciplinary professional societies. One program that may present an opportunity for collaboration is the Engineering with Nature® (EWN) program developed by the U.S. Army Corps of Engineers (USACE; Kurth et al. 2020). EWN has partnered with nonprofits such as The Nature Conservancy and its Natural Infrastructure Initiative, providing a model upon which recruitment and partnership efforts could be based. These partnerships also provide a unique opportunity for future collaboration on living shorelines projects; restoration practitioners can benefit from engineers' perspectives, and direct work with the USACE can help to streamline permitting and foster connections between coastal managers and engineering firms. Communications projects drawing attention to and building upon these existing resources could go a long way in generating interest and expanding training programs.

Two concerns raised in interviews with both engineers and policymakers were related to structural persistence of non-hardened structures and the efficacy of small-scale projects in achieving the desired restoration goals. Overall, these practitioners expressed a need for research that can provide data showing that nature-based approaches are both practical and feasible. First, engineers identified a need for projects documenting the ability of non-hardened structures to effectively persist over time and stabilize coasts, such as oyster restoration projects as a substitute for seawalls, or dune restoration using driftwood and cobble. Another area of interest among engineers interviewed was the influence of grain size on dune stability and vegetation growth and faunal communities. Second, both engineers and policymakers acknowledged that smaller-scale projects are considered valu-

⁴ Miller, J.K., Rella, A., Williams, A., Sproule, E., 2015. Living Shorelines Engineering Guidelines. Stephens Institute of Technology, 102 pp. Available from: http://stewardshipcentrebc.ca/PDF_docs/GS_LocGov/BkgrdResourcesReports/living-shorelines-engineering-guidelines.pdf. Accessed 29 January, 2022.

able within their field, but only so as they lead to larger-scale projects that increased the area of restoration. This perception may overlook the importance of these pilot projects for demonstrating efficacy and usefulness of nature-based infrastructure. Finally, a major knowledge gap emphasized in both literature and stakeholder interviews was a lack of direct green vs. gray comparisons. In Kurth et al. (2020), scientists recommended conducting formal tradeoff analyses comparing shoreline armoring with natural infrastructure; this was supported by several other sources indicating a need for longer-term data on stability, cost, and maintenance over time for both hard and soft defenses from sea level rise (Bragg et al. 2021; O'Shaughnessy et al. 2020; Sutton-Grier et al. 2018)⁵. Targeting these questions and research gaps can answer important questions within the field of natural resources engineering and establish guidelines for long-term design.

Continuing to Fund Long-Term Monitoring.—The most frequently identified need among all practitioners interviewed (57%) and supported strongly by literature sources (62%; Table 2) was increased funding for long-term monitoring especially for Pacific coast projects. Datasets of 10+ years are integral to understanding living shorelines' success from cross-disciplinary perspectives (Bayraktarov et al. 2016; Waltham et al. 2021). The primary goal of engineers is to create long-lasting structures, and insufficient data on persistence of living shorelines is a barrier in recruiting engineers to work with nature-based infrastructure. From a biological standpoint, ecological communities fluctuate both seasonally and annually, and long-term monitoring data is needed to distinguish restoration effects from interannual variation. Maintenance requirements and costs over time are also important information for property owners, local governments, and potential mitigation funders, and cannot be properly assessed without long-term studies. Given this need for long-term data and the research priorities identified in our study, priority should be given to projects assessing the structural persistence of nature-based infrastructure, with a standardized set of metrics such as elevation, habitat cover, ecological community responses, and maintenance requirements. Potential funders should seek out restoration efforts with concrete communications goals or with coordinated projects in other geographic locations.

Small foundations are integral to funding monitoring programs, especially in partnership with universities and local nonprofits. Having a long-term dataset was often referenced by practitioners when discussing scalability, as it provides more concrete evidence of the efficacy of living shorelines to planners and managers. Providing funding for unique monitoring approaches (i.e., community science programs, volunteer data collection days, video and drone monitoring) would be a creative strategy to increase visibility of living shorelines projects and to incorporate community engagement. Because long-term monitoring requires continual efforts to secure funding, leveraging funds in this way is a strategic approach that will contribute to larger-scale research and secure support for smaller organizations that may not have time or capacity to continually apply for short-term funding.

Investing in Communications & Media Projects.—Communications campaigns were also recommended by a majority of interviewees (12 practitioners or 52%; Table 2). This need was identified in the literature review as well, within 14 sources or 28% of the literature reviewed (Table 2). The strong relative emphasis on communications campaigns in interviews highlights a need that may be underrepresented in published literature. While

⁵ Myszewski, M., Alber, M., 2016. Living Shorelines in the Southeast: Research and Data Gaps. Athens, Georgia. 203 pp. Available from: <http://southatlanticalliance.org/wp-content/uploads/2016/09/Living-Shorelines-in-the-Southeast.pdf>. Accessed 29 January, 2022.

interviewees indicated that communication is progressing among scientists studying different habitats (dunes and oyster beds, for example) and between scientists and other professionals, improvement is still needed. Improved communication begins with better consensus on the definition of living shorelines themselves. The interdisciplinary nature of living shorelines work often means that different experts have different interpretations of which projects can be considered living shorelines. Scientists need to continue building a network to compile case studies regionally and to be aware of advances in disciplines not directly related to their own. The ability to compare methodologies, materials, coastal context, and successes or failures is needed for all types of living shorelines restoration (Baggett et al. 2015; Bayraktarov et al. 2016; Molino et al. 2020; Zeigler et al. 2021). Better communication is also especially needed in conveying the importance of living shorelines to the public (Bragg et al. 2021; Molino et al. 2020).

The ability of coastal communities to visualize proposed changes to their local beaches and properties is essential in gaining public support, whether it be through a projected image presented at a local meeting, a video project or virtual reality experience, or an example of a successful pilot project in a similar coastal context. Several practitioners suggested unique approaches to providing visualization for proposed or ongoing projects. Social media outreach is an important first step, and regularly providing educational information and project updates will shift public knowledge and perceptions. Other creative approaches to outreach include showing drone imagery of restored sites, models of proposed projects, or simulations of proposed changes on a phone app. These are all engaging examples of showing local communities, policymakers, and landowners how their local coastline will change and highlighting benefits of natural shorelines.

Communicating benefits to local management and communities is an important step in gathering more ground-up demand for living shorelines projects, and communications campaigns are particularly effective if economic incentives are highlighted. While the ecological value and ecosystem services of living shoreline restoration are important, it is essential to communicate the precise value of increased fish production, water quality improvements, wave attenuation, and other benefits. Projects translating ecosystem services into economic values, with specific plans for communicating these findings, should be considered for funding as they will drive increased demand and leverage future funds.

Prioritizing Community Engagement.—Practitioners agreed that community engagement is essential for living shorelines success and should continue to be a major focus in future restoration efforts. Increasing connection with local stakeholders, providing an outlet for them to express their concerns, keeping them informed about recent scientific findings, and demonstrating the services provided by restored habitats are all necessary steps in maintaining this connection. Community engagement has been identified to both increase stewardship of existing and restored habitats as well as to increase understanding and support for restoration and associated funding (Bragg et al. 2021; Chang et al. 2019; Scyphers et al. 2020)². Efforts to promote volunteer-based or community science also provide an opportunity for long-term data collection and increase engagement with restoration projects; such stakeholder engagement efforts have been shown to change the perceptions and views of community members regarding restoration projects (Boudreau et al. 2018; Josephs and Humphries 2018).

An increased focus on nature-based coastal resilience measures is especially needed in communities affected disproportionately by sea level rise and environmental degradation. In under-served communities, socioeconomic factors often limit access to environmental education and to nature itself. Public perception and community well-being can both be

supported through educational measures, such as field trips to restored beaches for students or discussions with local fishermen about increased fish presence post-restoration. Agencies such as the California State Coastal Conservancy (SCC) have been working in recent years to re-center their mission on environmental justice and equity; these efforts are needed in all regions to increase scalability of public interest and collaboration.⁶ Building ground-up demand for living shorelines projects starts with building a connection to projects within communities, then enhancing dialogue between communities.

Fostering connections between communities and government in joint projects can have lasting positive impacts, especially when local knowledge enhances resource management. This can be seen in the collaboration between indigenous communities and local government in community-based subsistence areas and biocultural resource management in Hawaii (Chang et al. 2019; Winter et al. 2018). Indigenous fish ponds in Hawaii serve as a valuable example of community-based adaptive management and cross-disciplinary collaboration. They highlight the importance of supporting indigenous group autonomy and enhancing productive relationships between stakeholders within a community.

Lessons Learned for Future Efforts.—Both the literature review and interview portions of this study reaffirmed a need for continued outreach and discussion with stakeholders in living shorelines restoration, including local community members and volunteers, homeowners, students, scientists, engineers, members of state agencies and nonprofit organizations, and more. Engaging with stakeholders, including coastal landowners and other community members, early in the restoration process allows for priorities and community values to be established early on and persist throughout all project stages (Fitzsimons et al. 2020; Josephs and Humphries 2018). Taking advantage of every opportunity possible in bringing people together and enhancing dialogue between communities, from meetings and webinars to accessible regional conferences, is essential (Bragg et al. 2021; Toft et al. 2017). Many interviewees were very enthusiastic about continuing stakeholder outreach efforts, emphasizing the importance of diverse perspectives in discovering opportunities in coastal communities. Increased representation is particularly needed moving forward for native communities and communities of color. These groups should form a substantial part of discussions on living shorelines, as their involvement is needed to address their concerns and promote projects that advance their interests and well-being.

Our research and conversations also highlighted a need for increased communication and data sharing among West coast living shorelines practitioners. Our interviews primarily incorporated perspectives from the Pacific coast; many interviewees from this region agreed that local restorations are heavily informed by work done in the Gulf and East coasts, where living shorelines research, especially involving oyster restoration, is older. Less is still known about restoring certain Pacific coast species such as the Olympia oyster, or addressing challenges within unique coastal settings (i.e., open coast, higher energy environments). Supporting demonstration projects on the Pacific coast in new coastal contexts, collecting long-term data, and sharing findings effectively among stakeholders are all essential next steps in increasing the scale of living shorelines restoration in the future.

⁶ California State Coastal Conservancy (SCC), 2020. State Coastal Conservancy JEDI Guidelines in Action. 5 pp. Available from: https://scc.ca.gov/files/2020/09/JEDI_Guidelines_In_Action_FINAL.pdf. Accessed 29 January, 2022.

Conclusions

After reviewing recent literature on living shorelines and gathering insight from a varied group of cross-disciplinary practitioners, the following priorities were identified: (1) supporting pilot or demonstration projects in their early research stages, (2) increasing availability of training programs for engineers utilizing nature-based infrastructure, (3) monitoring ecological and structural properties of living shorelines in the long-term (5+ years), (4) communicating results, project significance, and visual resources to stakeholders within and between communities, and (5) advancing the causes of environmental justice and equity.

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A New Maximum Length for the Bluebanded Ronquil, *Rathbunella hypoplecta* (Zoarcoidei: Bathymasteridae)

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Abstract.—Throughout 2019, multiple large *Rathbunella* specimens were collected from recreational hook and line fishermen off San Diego County, California, USA. The individuals were larger than the previously reported maximum size for *Rathbunella* and exhibited a dark coloration known but not usually depicted for the genus. Herein, we provide morphological and molecular evidence that these specimens are Bluebanded Ronquils, *Rathbunella hypoplecta*, and we increase the maximum size of the species to 242 mm standard length. We provide descriptions of the specimens and the dark-phase coloration and demonstrate that dark and light-phase individuals are not genetically different.

The bathymasterid genus *Rathbunella* Jordan and Evermann, 1896 is a relatively common demersal zoarcoid genus comprising two species found on the northeastern Pacific coast from Marin, California to San Carlos, Baja California (Love and Passarelli 2020). Of the two species, *Rathbunella alleni* Gilbert, 1904 is distributed along the full range, but is most common in central California from depths of 2–146 m, whereas *R. hypoplecta* (Gilbert, 1890) is known from Point Conception, California to Santo Tomás, Baja California from depths of 9–136 m (Stevenson and Matarese 2005; Love and Passarelli 2020). The two species have been confused with some authors considering *R. alleni* as a synonym of *R. hypoplecta* (Eschmeyer and Herald 1983); however, Stevenson and Matarese (2005) redescribed the species and identified a suite of characters supporting its validity and distinction from *R. hypoplecta*. *Rathbunella alleni* differs in having rougher scales, lacking an accessory preopercular pore, having males with large canines and having a blue stripe through the anal fin rather than blue bands and is generally considered to be rarer than *R. hypoplecta* (Miller and Lea 1972). *Rathbunella hypoplecta* is the larger of the two species, generally reported to reach a maximum size of 216 mm total length (TL) with *R. alleni* around 161 mm standard length (SL) (Love and Passarelli 2020).

During 2019, California Department of Fish and Wildlife (CDFW) personnel collected five *Rathbunella* specimens ranging from 248–277 mm TL (unpreserved) off San Diego County, California, USA, that are larger than the previously reported maximum length for the genus. Specimens were collected on hook and line by recreational anglers and recovered by CDFW personnel sampling from fishing operations. Additional material was observed but not retained from locations throughout San Diego County, CA. Historically, there has been some confusion on the maximum size of *R. hypoplecta*. Barnhart (1936) reported a

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Table 1. Morphometrics for newly collected *Rathbunella hypoplecta* specimens and those reported by Stevenson and Matarese (2005). Cephalic measurements are reported as percentage head length, all others are percentage standard length (SL). Means are in parentheses.

	New specimens	Steven and Matarese (2005) specimens
	<i>n</i> = 5	<i>n</i> = 11
Unpreserved Total Length	248–277	-
Preserved Total Length	245–276	-
Preserved SL	209–242	116–175
Head length	20.5–22.7 (21.5)	20.4–23.7 (21.6)
Snout length	24.4–27.6 (26.3)	16.5–27.0 (22.5)
Upper jaw length	36.1–40.7 (38.8)	29.6–38.6 (33.3)
Eye diameter	18.6–20.7 (19.8)	18.9–24.9 (22.5)
Interorbital width	14.0–17.1 (15.9)	10.8–15.4 (13.3)
Body depth at dorsal-fin origin	14.4–15.6 (14.8)	13.3–15.2 (14.3)
Body depth at anus	15.2–17.6 (16.2)	15.0–17.4 (16.0)
Caudal peduncle depth	6.9–7.4 (7.1)	7.2–8.6 (7.8)
Dorsal-fin length	76.2–79.9 (79.0)	76.8–80.8 (78.5)
Anal-fin length	54.1–57.5 (55.9)	51.3–57.9 (55.0)
Pectoral-fin length	16.3–17.9 (16.8)	15.7–18.8 (17.2)
Pelvic-fin length	10.5–12.1 (11.1)	10.2–13.1 (11.6)
Predorsal length	16.4–19.0 (17.3)	18.0–20.3 (18.9)
Preanal length	40.5–41.9 (41.0)	40.3–45.5 (42.2)
Snout to anus	39.3–40.6 (39.8)	38.3–42.8 (40.3)
Snout to pectoral-fin base	20.7–22.6 (21.9)	21.1–25.1 (22.6)
Snout to pelvic-fin base	18.9–21.1 (19.7)	14.9–23.0 (19.5)

maximum length to 8 inches (203 mm). Miller and Lea (1972) report a maximum total length (TL) for the genus *Rathbunella* as “around 8.5 in.” or 216 mm. Fitch and Lavenberg (1975) report the same maximum length of 216 mm, but state this may be a different species as the largest they personally observed was 159 mm. Eschmeyer and Herald (1983) report a smaller maximum size of 160 mm, likely from Fitch and Lavenberg (1975). Love (2011) reports 161 mm as the maximum size and Kells et al. (2016) report 8.5 in., likely following Miller and Lea (1972).

Stevenson and Matarese (2005) examined UCLA W60-64, 215 mm standard length (SL), which is potentially the source for the previous reported largest specimen; however, this is not recorded as the largest specimen in their study (Stevenson and Matarese 2005: Table 1). This specimen is now LACM 51837-1 and is remeasured as 213 mm SL and 245 mm TL (W. Ludt, pers. comm. 2019). Additionally, Stevenson and Matarese (2005) report the locality in error as “100 mi off Long Beach”, as the verbatim locality in the UCLA catalog is “Los Angeles Co.: off Long Beach, Horseshoe Kelp Bed”.

Based on morphological comparison, we determined that these large specimens represent *R. hypoplecta* and herein provide morphological and molecular support for our identification. We increase the maximum size for *R. hypoplecta* and the genus *Rathbunella* to 277 mm TL (unpreserved; Fig. 1A) and report this in Love and Passarelli (2020). Additionally, four of the five specimens collected exhibited dark coloration likely matching the “Deep-water Ronquil” a potential undescribed species mentioned by Eschmeyer and Herald (1983) with a darker overall body color and bright blue bars between pairs of anal-fin rays, while one specimen had the more common light coloration with a sandy body color with a bright blue stripe through the anal fin. Stevenson and Matarese (2005) suggested that the

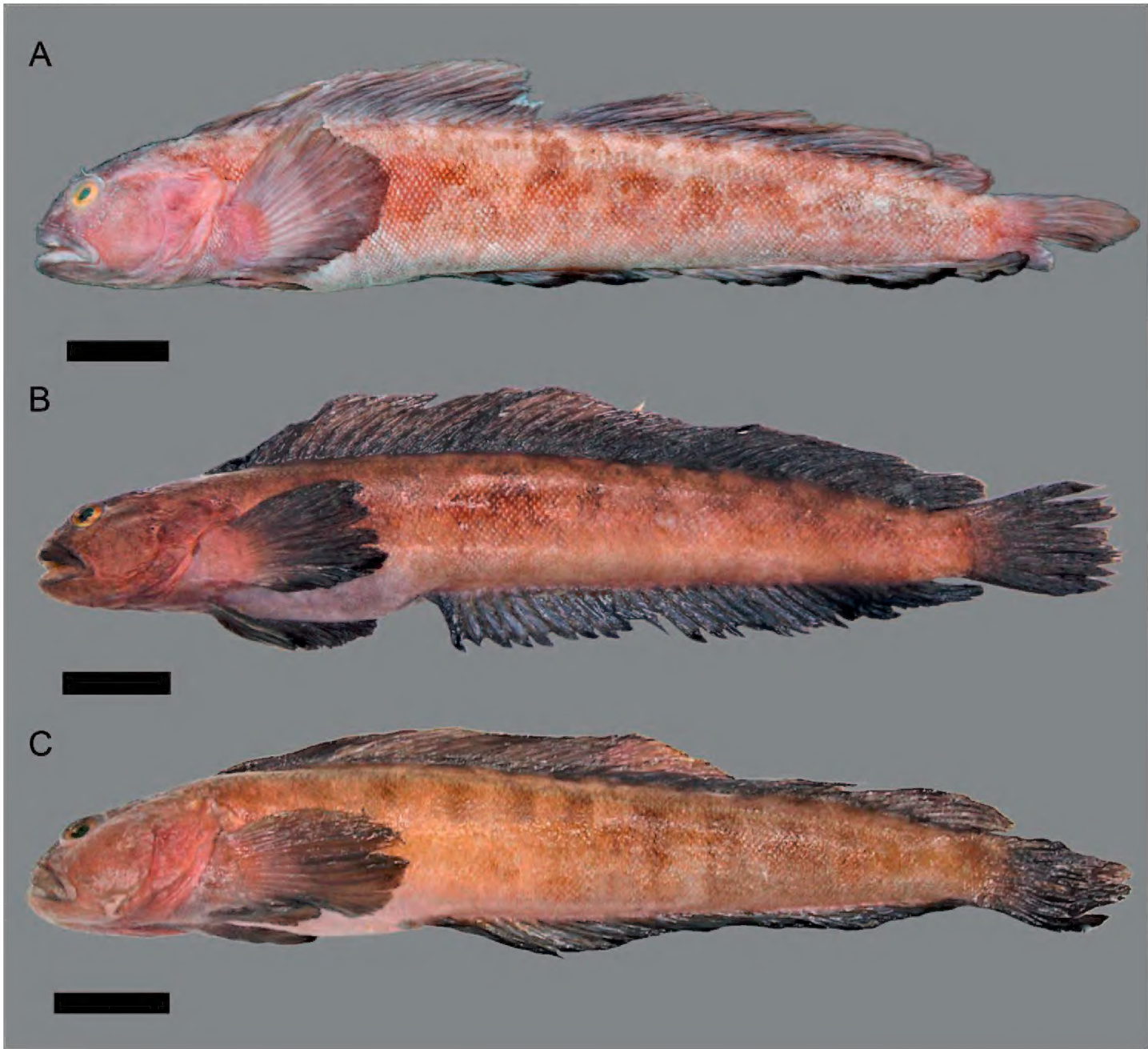


Fig. 1. Large specimens of *Rathbunella hypoplecta* collected in San Diego County, California, USA. A) SIO 19-56, 242 mm SL, largest individual, preserved; B) SIO 19-7, 217 mm SL, dark-phase coloration prior to preservation; C) SIO 19-57, 209 mm SL, light-phase coloration prior to preservation. Scale bars equal 20 mm.

“deepwater” specimens do not represent a separate species but a regular color phase for *R. hypoplecta*. We provide genetic comparisons of the dark and light color phase specimens and conclude these are indeed different color phases of the same species.

Materials and Methods

Methods of counting and measuring follow Hubbs and Lagler (1958) and Stevenson and Matarese (2005). Measurements were taken with digital calipers and are reported to the nearest 0.1 mm. Fin element and vertebral counts were taken from digital radiographs. Gill raker counts are presented as upper (epibranchial) + lower (ceratobranchial) rakers on the anterior face of the first arch; the angle raker is included in the second count. Recently collected specimens are compared with counts and measurements from Stevenson and Matarese (2005). Where counts were recorded bilaterally, both counts are given and separated from each other by a slash; the first count presented is the left count. Morphometric values for the specimens are presented in Table 1. Specimens examined and reported

are deposited in the Scripps Institution of Oceanography (SIO); institutional abbreviation follows Sabaj (2019).

To further verify species identity, mitochondrial cytochrome c oxidase subunit I (COI) sequences from the recent specimens were compared with publicly available sequences obtained from Genbank. PCR reactions were prepared to amplify an approximately 655 bp fragment of the cytochrome oxidase I (COI) mtDNA gene using an M13-tailed primer cocktail COI-3 from Ivanova et al. (2007). Reactions were prepared with a final concentration of 2 mM each dNTP, 0.25 μ M of each primer, 0.5 mg/ml Bovine Serum Albumin (BSA), and 0.5U of standard DNA polymerase. Following a denaturation at 94°C for two minutes, 35 cycles of the following cycling were performed: 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min. A final extension at 72°C for three min terminated the cycling. Di-deoxy Sanger sequencing was performed in both directions with universal M13 primers using BigDye Terminator v3.1 chemistry following manufacturer's protocol. Sequences were generated on an ABI3730 at the Southwest Fisheries Science Center. Sequences were aligned and edited in Sequencher v4.8, and the resulting contig was compared to sequences in GenBank using the BLAST function.

Results

Rathbunella hypoplecta (Gilbert, 1890)

Fig 1A–C, Table 1

Material.—*Rathbunella hypoplecta*, five specimens, 208.5–242 mm SL, San Diego County, California, USA: SIO 19-7 (Fig. 1B), 217 mm SL, 256 mm TL, Box Canyon, 33°19' 6"N, 117°31' 48"W, 78.6 m, hook and line, recreational fisherman, 25 March 2019; SIO 19-55, 236 mm SL, 271 mm TL, Devil's Reef, ca. 3.7 km (2.3 mi) off Leucadia, 33°03' 54"N, 117°31' 48"W, 85.4 m, hook and line, recreational fisherman, 5 May 2019; SIO 19-56 (Fig. 1A), 242 mm SL, 276 mm TL, Box Canyon, 33°19' 6"N, 117°03' 54"W, 72.1 m, hook and line, recreational fisherman, 9 May 2019; SIO 19-57 (Fig. 1C), 209 mm SL, 245 mm TL, Box Canyon, Devil's Reef, ca. 3.7 km (2.3 mi) off Leucadia, 33°03' 54"N, 117°20' 30"W, 87.1 m, hook and line, recreational fisherman, 18 June 2019; SIO 19-68, 225 mm SL, 261 mm TL, 3.2 km (2 mi) off Del Mar Fairgrounds, 32°58' 36"N, 117°18' 48"W, 69.5 m, hook and line, recreational fisherman, 4 October 2019.

Diagnosis.—A species of the genus *Rathbunella* differentiated from its congener, *R. aleni*, by the following combination of characters: ctenoid scales weak with short cteni (vs. long); preopercular pores 7 + 1 (vs. 7); first dorsal-fin pterygiophore inserted between first and second neural spine; anal-fin membrane solid bright blue, sometimes in bands (vs. a single stripe).

Description.—D V–VI, 40–41 (45–46 total elements); A II, 32 (34 total elements); P 15–17/16–17; Vertebrae 14 + 36 (one with 34), total 50 (one with 48); upper procurrent caudal-fin rays 7; upper principal caudal-fin rays 7 (one with 6); lower principal caudal-fin rays 6; lower procurrent caudal-fin rays 6 (one with 7); pored lateral-line scales 83–85. Head rounded, cheek with scales; snout rounded, blunt, 24.4–27.6% HL; eye diameter 18.6–20.7% HL; interorbital width 14.0–17.1% HL; jaws subequal, lips thick; mouth slightly oblique and short, not extending past midorbit, upper jaw length 36.1–40.7% HL; teeth in upper and lower jaw small, conical, some slightly recurved, a few larger teeth, no canines; small teeth on vomer and palatine; gill rakers stout with tooth patches, 3–4 + 10–11; branchiostegal membranes joined broadly and free from isthmus. Preopercular lateralis canal with 7 pores plus a single pore from secondary caniculus off anteroventral end of

main canal; mandibular canal with 4 pores. Scales weakly ctenoid, over entire body except just anterior dorsal-fin, head anterior of cheek and on fin membranes except dorsal and caudal-fin bases. Lateral line originating posterior to opercular margin and terminating before dorsal-fin insertion.

Coloration when fresh.—Four of the five retained specimens displayed a dark color phase (Fig. 1A–B), likely the “Deepwater Ronquil” of Eschmeyer and Herald (1983), whereas one (SIO 19-57; Fig. 1C) displayed the more typical light sandy color phase reported in *Rathbunella hypoplecta* (Love 2011). The dark-phase specimens are generally dark blue-black with indistinct lighter barring along the body. When fresh, anal fin with iridescent royal blue bars nearly touching to give solid blue appearance to fin, fading to dark gray-black in death (Fig 1B), no red bars evident between anal-fin membranes as described in Eschmeyer and Herald (1983), tips of anal-fin rays black. The light color phase sandy beige to grey ground color with more distinct darker barring along the body and base of dorsal fin; dorsal fin dark grey-blue; when fresh, anal fin with bright royal blue stripe down length covering distal two-thirds of fin fading to dark grey-black in death, tips of anal-fin rays black (Fig. 1C).

Coloration in alcohol (Fig. 1A).—Dark and light-phase specimens similar in preservation. Head dark brown dorsally, cheek reddish-brown; body tan in ground with dark brown chain extending along midbody to caudal peduncle; 10–12 indistinct dark brown blotches on dorsal surface, extending into dorsal-fin element bases, blotches 2–3 elements long. Dorsal fin dark gray becoming brown distally; anal fin light gray, rays light brown, distal tips of anal-fin rays distinctly black; pectoral fin light gray becoming dark distally; pelvic fins blue-black; caudal fin brown with thin dark margin.

Habitat.—These specimens were collected by recreational fishermen bottom-fishing for rockfishes (*Sebastes*) on deep rocky reef habitats 3.2–4.8 km (2–3 mi) offshore in 69.5–87.1 m of water.

Discussion

We resolved 649–652 bp of the COI gene for two specimens: SIO 19-7 and SIO 19-57 (GenBank Accession numbers: OP480876 and OP480877, respectively). BLAST results recover 99.85–100.00% and 99.69–99.85%, respectively, matches of these sequences to vouchers identified as *Rathbunella hypoplecta* on Genbank: EU400166 and GU440493, which correspond to voucher SIO 03-60, and KF930350, corresponding to tissue KUT 494 and voucher KU 28137. The next closest sequence is 88.89–88.99% similar (FJ165465) and identified as *Xiphister mucosus* in Stichaeidae. Interestingly, this is more similar than sequences from other bathymasterid genera. Unfortunately, no public COI sequence exists for *R. allenii* for intrageneric comparison. We also found that the COI sequences were 99.85% identical between the light-phase (SIO 19-57; Fig. 1C) and dark-phase specimens (SIO 19-7; Fig. 1B) providing additional evidence that the dark-phase specimens do not represent a distinct species.

Morphometrics and meristics are reported in Table 1. All five specimens fall within the meristic and morphometric ranges reported for *R. hypoplecta* by Stevenson and Matarese (2005) except some morphometrics just outside the range (Table 1). In the new specimens, we found a slightly longer snout length (27.6 vs. 27.0% HL) and upper jaw length (40.7 vs. 38.6% HL), a slightly smaller eye diameter (18.6 vs. 18.9% HL), a wider interorbital width (17.1 vs. 15.4% HL). In body measurements, we identified a slightly deeper body at dorsal-fin origin (15.6 vs. 15.2% SL), a slightly shorter dorsal-fin base (76.2 vs. 76.8%

SL), a slightly narrower caudal peduncle depth (6.9 vs. 7.2% SL) and a shorter snout to pectoral-fin base (20.7 vs. 21.1% SL). The new specimens differ from *R. alleni* in having weakly ctenoid body scales (vs. strong), an accessory preopercular pore and the first dorsal-fin pterygiophore inserted between the first and second neural spine (vs. anterior or dorsal to the first neural spine).

Conclusions

This new material increases the known maximum size for the genus *Rathbunella* from 216 mm SL to 242 mm SL (276 mm TL) in preservation and 277 mm TL unpreserved. Additionally, we used genetic comparisons of this material to determine that the potentially undescribed “Deepwater Ronquil” of Eschmeyer and Herald (1983) is in fact just the dark-phase coloration of *Rathbunella hypoplecta*.

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